

Mohamed Fawzy Ramadan  
Tamer E. Moussa Ayoub  
Sascha Rohn *Editors*

*Opuntia* spp.:  
Chemistry,  
Bioactivity  
and Industrial  
Applications

# *Opuntia* spp.: Chemistry, Bioactivity and Industrial Applications

Mohamed Fawzy Ramadan  
Tamer E. Moussa Ayoub • Sascha Rohn  
Editors

# *Opuntia* spp.: Chemistry, Bioactivity and Industrial Applications

 Springer

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*Dedicated to **Professor Lothar W. Kroh***



*Prof. Kroh is a German chemist. He was a Professor of Food Chemistry at the Technical University of Berlin from 1993 to 2019. Prof. Kroh investigated the molecular basis of color formation in food (especially with a focus on the caramelization and the MAILLARD reaction) and properties of sugar degradation products. He further studied structural-effect relationships of secondary plant metabolites such as phenolic compounds. From 2009 to 2012, Prof. Kroh was Dean of the Faculty of Process Sciences of the TU Berlin. In 2019, Prof. Kroh was awarded the Joseph-König-Gedenkmünze*

*that is given by the German Society of Chemists for excellent research and academic developments in food chemistry. This book is dedicated to Prof. Kroh, because he supervised all three editors and gave them the opportunity to develop academically and create a career with two of the editors being holder of research chairs in their universities. At the time they worked together, they came first into contact with cactus pear research that is still ongoing today. We highly appreciate this support. Thank you!*

*Mohamed Fawzy Ramadan  
Tamer E. Moussa Ayoub  
Sascha Rohn*

# Description

Consumption of exotic and nontraditional fruits, rich in health-promoting phytochemicals, may expand consumers' life span. Plants of *Opuntia* spp. are able to provide promising fruits and further products. *Opuntia* plants are commonly distributed in the arid and semiarid regions of the world. Concerning global climate change, *Opuntia* spp. were suggested by the FAO to be a promising and strategic crop in different regions, especially those that suffer from a lack of water. The ecological advantages of *Opuntia* spp. allow uptake of CO<sub>2</sub> at night, and consequently, minimizing water loss during photosynthesis. Many *Opuntia* spp. are referred to as notorious weeds in some parts of the world, and some species, such as *Opuntia ficus indica*, are commercially cultivated for food and feed applications.

*Opuntia* spp.: *Chemistry, Bioactivity and Industrial Applications* covers specific topics with a focus on cultivation and composition of *Opuntia* spp. as well as their chemistry, technology, functionality, and applicability of *Opuntia* spp. fruits, extracts, and by-products.

Edited by a team of experts, *Opuntia* spp.: *Chemistry, Bioactivity and Industrial Applications* brings together a diversity of food science developments to scientists, students, and interested consumers and combines disciplines such as chemistry, nutrition, agriculture, food science, pharmacology, cosmetics, and the technology-related disciplines.

*Opuntia* spp.: *Chemistry, Bioactivity and Industrial Applications* is an essential textbook for pharmaceuticals, nutraceuticals, and functional, as well as traditional food developers and research and development (R&D) managers, working in all sectors that use fruits, natural products, and medicinal plants. It is a useful reference work for companies reformulating their present product portfolio or developing new products.

## Key features

- Broad coverage encompasses cultivation, chemistry, technology, functionality, and applications of *Opuntia* spp.
- Authored by international scientists and industry experts.

- Addresses the growing application areas including pharmaceuticals, novel food, feed, nutraceuticals, and cosmetics.

**Readership**

- Researchers and students with a research interest in the area (pharmacologists, food chemists, food technologists, and agronomists).
- Developers of pharmaceuticals, cosmetics, and functional food, and R&D managers working in all sectors that use fruits, medicinal plants, and specialty oils.



# Preface

*Opuntia* spp. are rich in phytochemicals and health-promoting compounds that are believed to expand consumers' life span. The fruits, commonly known as cactus pears or prickly pears, are produced by *Opuntia* species (plant family Cactaceae). *Opuntia* plants are distributed in the arid and semiarid regions. The crassulacean acid metabolism occurring in these plants is responsible for their comparatively high water use efficiency compared to either C3 or C4 plants. Some *Opuntia* spp. are referred to as notorious weeds, and some species, such as *Opuntia ficus indica*, are grown for both food and feed uses.

*Opuntia* fruits are popular due to their nutritive value and health benefits, including antioxidant, antiulcerogenic, and antiatherogenic traits and the protective effects against LDL oxidation. Health-promoting traits are attributed to the high levels of phenolic compounds, betacyanins, betalains, and vitamins. Some *Opuntia* fruits have a high level of pigments (red to violet betacyanins and yellow to orange betaxanthins), which can be exploited as natural food colorants. Furthermore, *Opuntia* fruits contain a high amount of plant mucilage, serving as a thickening agent. Low acidity makes most of *Opuntia* fruits ideal ingredient for dairy products. Readily absorbable sugars, high vitamin C and mineral content, as well as a high total phenolic content, and the pleasant flavor make *Opuntia* an advantageous source for healthy and even novel food.

Cactus fruits are traditionally used for the preparation of several food products such as juices and jams. Moreover, *Opuntia* cactus plants are exploited for feeding animals. The commercial production of food and non-food products from *Opuntia* has been established in Mexico, the USA, and the Mediterranean countries. On the other side, by-products produced during the processing of *Opuntia* fruits remain a rich source of phytochemicals. The recovery of value-added compounds from the *Opuntia* by-products provides benefits by addressing sustainability and societal health management.

This book aims at creating a multidisciplinary forum for discussion on *Opuntia* spp. plants and products, in particular, with emphasis on cultivation, post-harvest, chemistry, functionality, health-promoting properties, and processing. The impact of traditional and innovative processing techniques on the recovery of high

value-added compounds from *Opuntia* spp. fruits and by-products is reported. Moreover, chapters of this book will discuss the potential applications of *Opuntia* spp. in food, feed, cosmetics, and pharmaceutical products.

Intending to provide major reference work for those of the scientific community involved in horticulture, food science, pharmacology, nutrition, and the oil industry, as well as for undergraduate and graduate students, this volume presents a comprehensive review of the aspects and results that have led to the advancements in *Opuntia* spp. chemistry, technology, and product development. We hope that the book will be a valuable source for people involved in all kinds of related disciplines.

We sincerely thank all authors for their valuable contributions and their cooperation during book preparation. The help and support given to us by the Springer-Nature staff, especially *Daniel Falatko* and *Arjun Narayanan*, was essential for completing our task and is highly appreciated.

**“Let food be your medicine and medicine be your food” (Hippocrates)**

Zagazig, Egypt  
Ismailia, Egypt  
Berlin, Germany  
April 2021

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## About the Editors



**Mohamed Fawzy Ramadan** born 1974, is a full professor of Biochemistry in the Agricultural Biochemistry Department, Faculty of Agriculture at Zagazig University (Egypt). Since 2013, Prof. Ramadan is a Professor of Biochemistry and the consultant of international publishing at the Deanship of Scientific Research (Umm Al-Qura University, Makkah, Saudi Arabia). Prof. Ramadan obtained his Ph.D. (*Dr. rer. nat.*) in Food Chemistry from Technische Universität Berlin (Germany, 2004). Prof. Ramadan continued his postdoctoral research in ranked

universities/research institutions in different countries such University of Helsinki (Finland), Max-Rubner Institute (Germany), Technische Universität Berlin (Germany), and University of Maryland (USA). In 2010, he was appointed to be Visiting Professor (100% research) at King Saud University. In 2012, he was appointed to be visiting Professor (100% teaching) in the School of Biomedicine, Far Eastern Federal University (Vladivostok, Russian Federation). Prof. Ramadan published more than 250 research papers and reviews in international peer-reviewed journals and several books and book chapters (Scopus *h*-index is 40 and more than 5000 citations). He edited and published several books—published by prestigious publishers (Springer, Elsevier, and CRC Press-Taylor & Francis)—in the field of food science and biochemistry. He was an invited speaker at several international conferences. Since 2003, Prof. Ramadan served as a reviewer for about 200 international journals and as editor of several highly cited international journals such as the *Journal of Medicinal Food* and *Journal of Advanced Research*. Prof. Ramadan received Abdul Hamid Shoman Prize for Young Arab Researcher in Agricultural Sciences (2006), Egyptian State Prize for Encouragement in Agricultural Sciences (2009), European Young Lipid Scientist Award (2009), AU-TWAS Young Scientist National Awards (Egypt) in Basic Sciences, Technology and Innovation (2012), TWAS-ARO Young Arab Scientist (YAS) Prize in Scientific and Technological Achievement (2013), and Atta-ur-Rahman Prize in Chemistry (2014).





**Tamer E. Moussa Ayoub** is a lecturer in Food Science at Food Technology Department, Faculty of Agriculture, Suez Canal University (Egypt). Dr. Tamer Moussa Ayoub obtained his Ph.D. from Technische Universität Berlin (Germany). His research was about identifying some bioactive compounds (mainly phenolic compounds) in cactus cladodes and fruits. He investigated the impact of thermal treatments and innovative nonthermal treatments like pulsed electric field (PEF) technology and high hydrostatic pressure (HHP) on the bioactive compounds in cactus fruits' juice. His research was also about the impact of

different processing technologies on different cactus fruit products. He is the first author and coauthor of several original publications that dealt with cactus fruits and cladodes. He shared his results in several international scientific conferences.



**Sascha Rohn** born 1973, is a full professor of Food Chemistry at the Technische Universität Berlin (Germany). He graduated from the University of Frankfurt/Main, Germany, with the first and second state examination in Food Chemistry, 1999. In 2002, he obtained his Ph.D. in Food Chemistry from the Institute of Nutritional Science, University of Potsdam, Germany, working on interactions of polyphenols with food proteins. After 2 years as a post-doctorate, he left Potsdam toward Berlin, where he did a habilitation at the Institute of Food Technology and Food Chemistry of the Technische Universität Berlin. From

October 2009 to October 2020, he was a full professor of Food Chemistry at the Hamburg School of Food Science, Institute of Food Chemistry, University of Hamburg (Germany). His group is dealing with the analysis of bioactive food compounds and their antioxidant activity. Especially, they are dealing with the reactivity and stability of bioactive compounds. The aim is to identify degradation products that serve as quality parameters, as process markers during food/feed processing, or as biomarkers in nutritional physiology. In this context, interactions of secondary plant metabolites with food proteins are the main research topic, where they use high-performance thin-layer chromatography as an alternative analytical tool. Results of their work have been presented in more than 200 publications so far (Scopus *h*-index is 50 and more than 6000 citations). More than 30 well-known scientific journals ask Prof. Rohn regularly to review scientific manuscripts. From 2006 to 2012, he was chairman of the Northeastern branch of the *German Food Chemical Society* (LChG). From 2014 to 2018, he was a member of the steering committee of the *German Nutrition Society* (DGE). In April 2015, he became a director of the *Institute for Food and Environmental Research* (ILU e.V.) in Potsdam-Rehbruecke, Potsdam, Germany. As a nonprofit organization, this institute conducts

applied, technologically oriented research and development for the food industry and preserving the environment. The organization pursues objectives that are exclusively and directly exploitable for everyday use. Members of the organization are companies from the food industry and tangential areas and representatives of non-university research.

# **Part I**

## **General Aspects**

# Chapter 1

## Introduction to *Opuntia* spp.: Chemistry, Bioactivity and Industrial Applications



Mohamed Fawzy Ramadan , Tamer E. Moussa Ayoub ,  
and Sascha Rohn 

**Abstract** *Opuntia* fruits, known as prickly pears or cactus pears, are produced by different *Opuntia* species (family Cactaceae). In the last two decades, food scientists and technologists showed increasing interest in plants of *Opuntia* spp., as they are rich in nutritive ingredients and bioactive constituents such as fibers, vitamin C, phenolic compounds, vitamin E, amino acids, minerals, and natural pigments such as betalains. *Opuntia* phytochemicals are believed to exhibit antiatherosclerotic, antioxidant, anticancer, and hepatoprotective traits. Therefore, the consumption of fresh or processed *Opuntia* fruits offers numerous health-promoting effects. On the other side, processing of *Opuntia* fruits generates a vast amount of by-products (peel, pulp, and seeds) that still are rich in bioactive constituents. This book aims to be a basis for a multidisciplinary discussion on the advances and developments of *Opuntia* cultivation practices, phytochemistry, food chemistry and technology, pharmacology, by-products valorization, and human nutrition as well as feed and non-food applications. The book contains several chapters under the following main sections: (1) *Opuntia* spp. metabolism, biodiversity, species and cultivars, (2) chemistry, functionality, and health-promoting properties of *Opuntia* spp., (3) technology and processing of *Opuntia* spp., and (4) food, feed, and non-food applications of *Opuntia* spp.

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**Keywords** Cactaceae · Zero hunger · Cactus pear · Prickly pear · Functional food · Market · Food technology · Food chemistry

## 1 *Opuntia*: Chemistry, Bioactivity, Technology, Functionality and Industrial Applications

In 2015, United Nations *Sustainable Development Goals* (SDG) were announced (<https://sustainabledevelopment.un.org>). The United Nations SDG comprise a vision of a peaceful, fairer, and sustainable world. In the food sector, all aspects of the value-added chain, including the way crops are cultivated, transported, processed, marketed, stored, and consumed, seem to be the essential link between people and the path to the development of sustainable economic. The second United Nations SDG (<https://sdgs.un.org/goals/goal2>), being called “Zero Hunger” aims (1) to end the hunger and ensure access to safe, nutritious, and sufficient food, (2) to end malnutrition, and address the nutritional needs for all people around the year, (3) to ensure sustainable implement resilient agricultural practices and food production systems that increase production, that help maintaining ecosystems, (4) to increase the agricultural production and income of small-scale farmers and food producers, and (5) to maintain the genetic diversity of cultivated plants, seeds, and domesticated animals and their related wild species. Besides, the third United Nations SDG, which is called “Good Health and Well-Being” aims to promote a healthy life and human well-being related to using of health-promoting crops and environmentally-friendly techniques in food processing (<https://sdgs.un.org/goals/goal3>). Food scientists and technologists are searching for unique food products with health-beneficial effects that might be designed for improving a long life. Recent research will significantly impact the way we eat in the near future (McClements, 2019; Ramadan, 2021).

The consumption of exotic and non-traditional fruits, rich in health-beneficial phytochemicals, may expand consumers’ life span. *Opuntia* spp. are plants distributed in the arid and semi-arid regions worldwide. *Opuntia* spp., commonly known as cactus pears or prickly pears, are promising plants produced by different *Opuntia* species (family Cactaceae). The crassulacean acid metabolism occurring in *Opuntia* plants is responsible for their comparatively high water-use efficiency compared to either C3 or C4 plants. Concerning global climate change, *Opuntia* spp. were suggested by FAO as promising and strategic crops in different regions of the world, especially those that suffer from a lack of water. The ecological advantages of *Opuntia* spp. allow uptake of CO<sub>2</sub> at night, and consequently, minimizing water loss during photosynthesis. The different colours (i.e., red, violet, green, and yellow) of *Opuntia* spp. are due to genetic variability (Barba et al., 2017). In some parts of the world, many *Opuntia* species are referred to as notorious weeds, and some species such as *Opuntia ficus indica*, are commercially grown for food as well as feed applications (Moussa-Ayoub et al., 2011, 2016, 2017; Barba et al., 2017; Surup et al., 2021).

Though *Opuntia* originated and being still extensively cultivated in Mexico, *Opuntia* plants are also cultivated in other countries, including Mediterranean countries, India, the USA, and South Africa. Numerous ingredients and bioactive compounds that show pharmacological properties have been identified in *Opuntia*, including vitamins, carbohydrates, lipids, minerals, and proteins with their specific amino acids. Those bioactive constituents provide antioxidant, anticancer, immunomodulatory, analgesic, cardiovascular supportive, antiepileptogenic, antidiabetic, antischistosomiasis, antiinflammatory, gastroprotective, hepatoprotective, and nephronprotective effects (Ramirez-Rodriguez et al., 2020). Health-promoting traits are mainly attributed to the high antioxidant levels resulting from the presence of phenolic compounds, betacyanins, betalains, and selected vitamins being found in high contents in the *Opuntia* fruits (Rahimi et al., 2019; Gouws et al., 2019). Besides, *Opuntia* fruits contain specific amino acids (i.e., taurine) and minerals, including iron, potassium, magnesium, calcium, sodium, and phosphorus. The *Opuntia* fruit consists of a juicy pulp (30–60% of fruit weight) with many small seeds (2–10% of fruit weight) surrounded by a thick peel (40–60% of fruit weight). The fruit pulp is the basis for producing different processed food products such as juices, while seeds have a high oil content. *Opuntia* seed oil is commonly used in different foodstuffs, cosmetics, and pharmaceutical products (Ramadan and Mörsel, 2003a, b; Barba et al., 2020). Some *Opuntia* fruits have a high level of natural pigments (red to violet betacyanins and yellow to orange betaxanthins), which can be exploited as natural food colorants. Furthermore, *Opuntia* fruits contain a high amount of unique plant mucilage, serving as a thickening agent. Low acidity makes most of *Opuntia* fruits such as *O. ficus-indica* fruits ideal for being added to different dairy products. Readily absorbable sugars, high contents of vitamin C, minerals, and phenolic compounds, as well as the pleasant flavour make *Opuntia* a pre-requisite source for developing functional and novel food (Ramadan and Mörsel, 2003a, b; Moussa-Ayoub et al., 2011, 2016, 2017; Barba et al., 2017, 2020).

*Opuntia* fruits can be intensively exploited for different food processing applications due to the high health-related values and functional traits. Moreover, the *Opuntia* cactus plants are exploited for feeding animals in different countries such as Tunisia, South Africa, and Latin Americas. Some countries like Peru cultivate *Opuntia* spp. to grow the cochineal (*Dactylopius coccus*) insect and produce the natural dye carmine. The commercial production of food and non-food products from *Opuntia* has been established in Mexico, the USA, and several Mediterranean countries (Jimenez-Aguilar et al., 2015; Barba et al., 2017, 2020).

Several food technologies, including thermal treatment, is applied for extending the shelf-life of *Opuntia* products. The thermal processing might cause a degradation of some thermolabile constituents and a change in the organoleptic traits. Therefore, applying innovative processing technologies such as pulsed electric fields (PEF), high-pressure homogenization (HPH), high-pressure processing (HPP), and ultrasound (US) can help retain (or release) bioactive compounds. On the other side, the vast amount of by-products produced during the processing of *Opuntia* fruits still could be utilized as a source of bioactive constituents. The recovery of value-added constituents from the *Opuntia* by-products provides benefits by addressing sustainability and societal health (Moussa-Ayoub et al., 2011, 2016, 2017; Barba et al., 2017).

## 2 *Opuntia* spp. Market

Due to the rapidly increasing world population and with its consumption needs, *Opuntia* has a remarkable international market. *Opuntia* fruits are cultivated mainly in Mexico with 428,300 t/year (44% of the World's production). Besides, *Opuntia* plants are produced in different parts of the world, including Canada, Mediterranean countries, the USA, and South Africa (Jimenez-Aguilar et al., 2015; Barba et al., 2017). In 2020, the top exporter of prickly pear was Canada with an export value of USD 317 M (16.4%), followed by the United States (USD 170 M, 8.8%), Chile (USD 123 M, 6.5%), Peru (USD 116 M, 6.4%), and Mexico (USD 107 M, 5.5%) ([www.tridge.com](http://www.tridge.com)). The top importer of prickly pear was the USA with an import value of USD 706 M (36%), followed by Germany (USD 298 M, 15%), Japan (USD 160 M, 8%), UK (USD 121 M, 6%), and Canada (USD 98 M, 5%).

## 3 *Opuntia* spp. in the International Literature

*Opuntia* spp. significantly attracts national and international scientific research. Thousands of articles were published on *Opuntia*. A survey and literature search with the keyword “*Opuntia*” in the database *PubMed* (March 2021) revealed 1155 published documents belonging to *Opuntia* cultivation, bioactivity, phytoextracts, amino acids, seed oil, fatty acids, bioactive constituents, and industrial applications. When *Opuntia* was searched as a keyword in the database *Web of Science*, almost 4000 documents were found (till March 2021), including articles (3400), proceedings paper (256), and review articles (140).

A careful search on *Opuntia* in the database *Scopus* showed that the number of documents published on *Opuntia* is high (approx. 4300 till March 2021) ([www.scopus.com](http://www.scopus.com)). Apart from the published documents, approx. 3750 were research articles, 277 conference papers, 159 reviews, and 35 book chapters. Figure 1.1 presents the document counts on *Opuntia* from the period 2000–2020. The documents annually published on *Opuntia* are significantly increased from 48 contributions in 2000 to 328 contributions in 2020. This measurable indicator reflects the importance and interest in *Opuntia* as a topic in the scientific community. Figure 1.2 presents the distribution of the types of documents on *Opuntia* in the period 2000–2020, which includes research articles (3121), conference papers (258), and review articles (159). The documents are related to the subject areas (Fig. 1.3) of Agricultural and Biological Sciences (39%), Biochemistry, Genetics, and Molecular Biology (10%), Environmental Science (9%), (Food) Chemistry (6%), Medicine (6%), and Pharmacology, Toxicology, and Pharmaceutics (6%).

Scientists from Mexico, USA, Brazil, Italy, Tunisia, Spain, India, South Africa, and Morocco emerged as main authors (Fig. 1.4). Journals with highest numbers of contributions were *Acta Horticulturae*, *Journal of the Professional Association for*

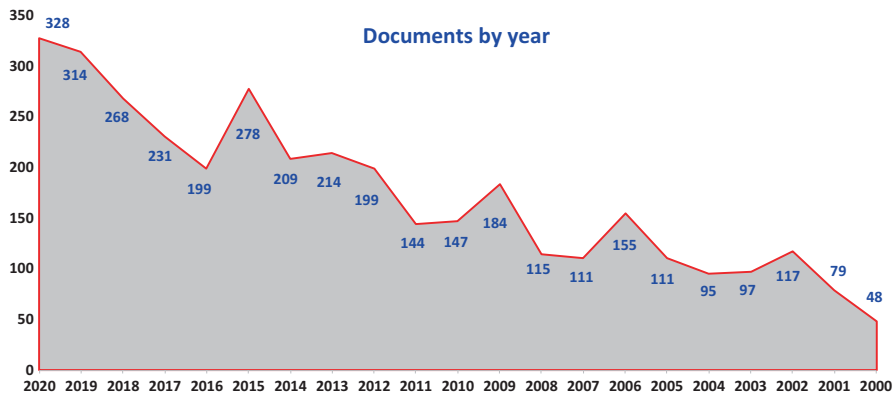


Fig. 1.1 Scholarly output on *Opuntia* from 2000 to 2020 ([www.scopus.com](http://www.scopus.com))

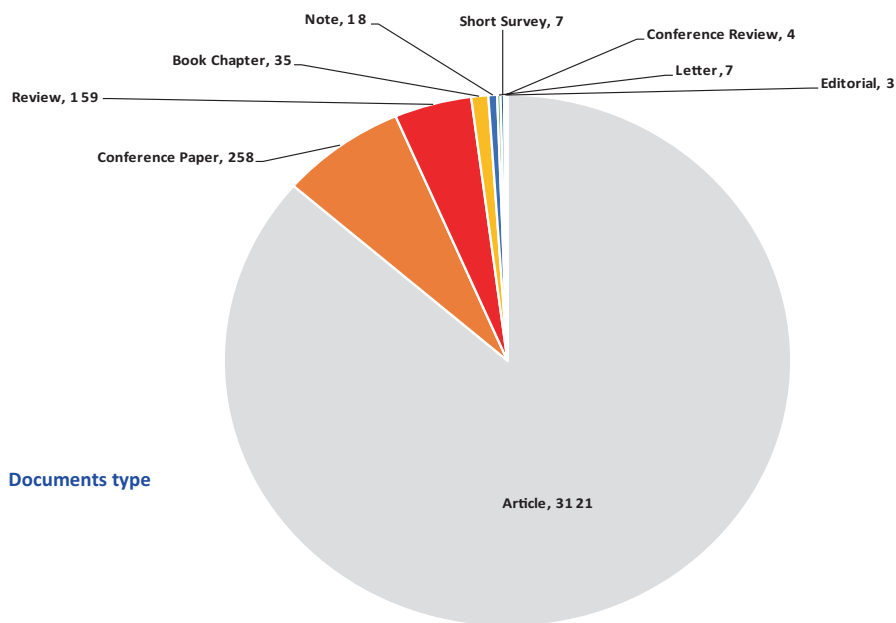
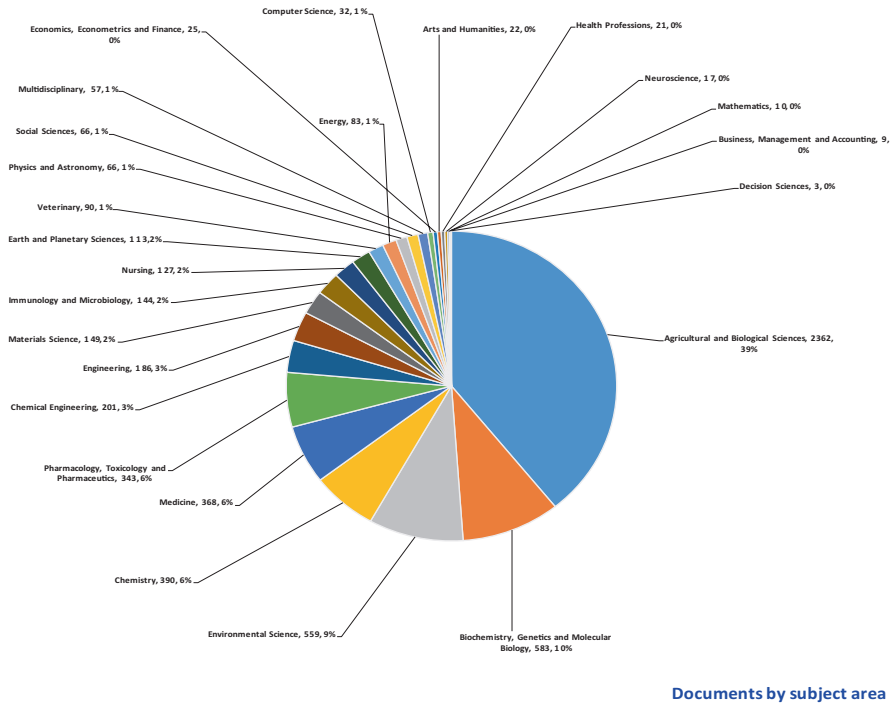


Fig. 1.2 Distribution by types of the document on *Opuntia* from 2000 to 2020 ([www.scopus.com](http://www.scopus.com))

Cactus Development, Journal of Ethnopharmacology, Journal of Arid Environments, Florida Entomologist, Food Chemistry, Revista Brasileira de Zootecnia, Haseltonia, Journal of Agricultural and Food Chemistry, Food Research International, Carbohydrate Polymers, and Industrial Crops and Products (Fig. 1.5).





**Fig. 1.3** Distribution by subject area of documents on *Opuntia* from 2000 to 2020 ([www.scopus.com](http://www.scopus.com))

## 4 Aims and Features of This Book

In the scientific literature, it is hard to find a handbook reporting on *Opuntia* cultivation, chemistry, and functionality. This handbook aims to be a basis for a multidisciplinary discussion on *Opuntia* spp. with particular emphasis on its horticulture, postharvest, marketability, chemistry, functionality, health-promoting properties, technology, and further processing. The effect of traditional and innovative processing on the recovery of functional compounds from *Opuntia* spp. wastes and by-products is reported, as well. Besides, the book discusses the potential applications of *Opuntia* spp. in food, cosmetic, and pharmacology.

The chapters provide a diversity of developments in the areas of food science and horticultural research. The editors invited highly-cited researchers for writing high-quality chapters with the major purpose of letting the readers of this book know more about this specific area of science. The book contains comprehensive chapters under the following main sections:

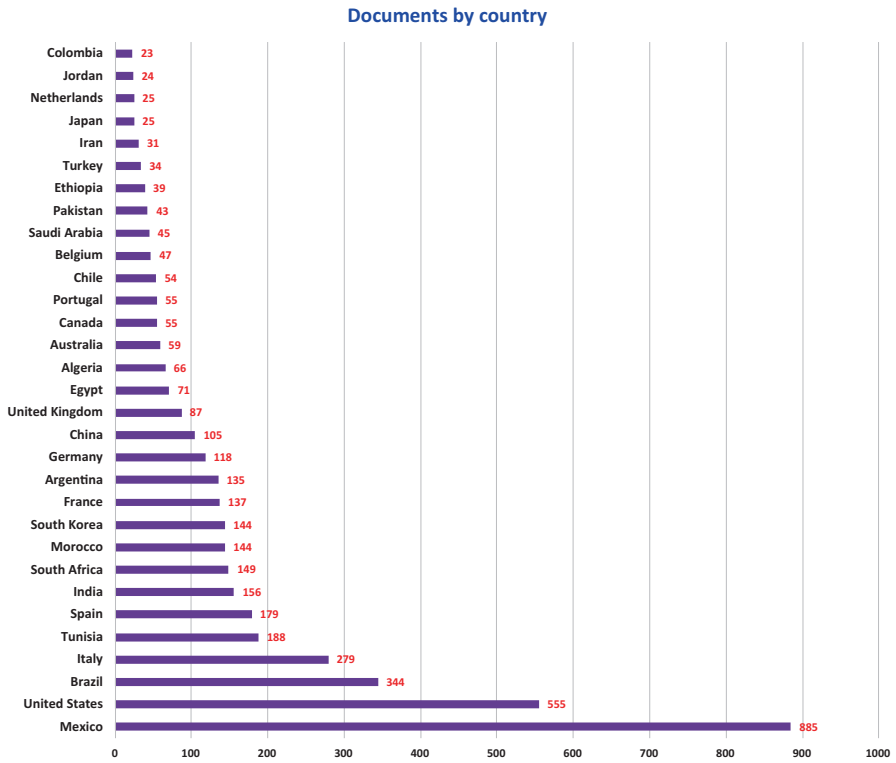


Fig. 1.4 Distribution by country of documents on *Opuntia* from 2000 to 2020 ([www.scopus.com](http://www.scopus.com))

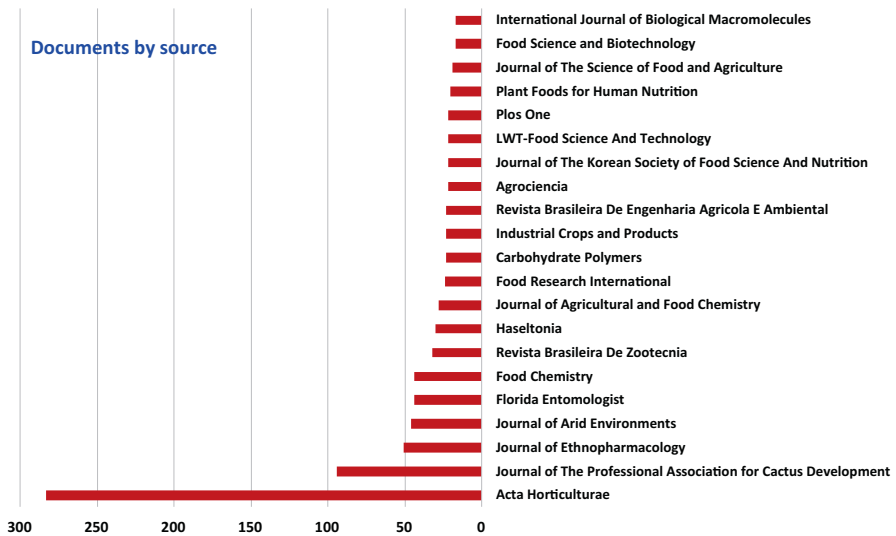


Fig. 1.5 Distribution by source title of documents on *Opuntia* from 2000 to 2020 ([www.scopus.com](http://www.scopus.com))

*Section 1: Opuntia* spp. metabolism, biodiversity, species and cultivars.

*Section 2: Chemistry, functionality, and health-promoting properties of Opuntia* spp.

*Section 3: Technology and processing of Opuntia* spp.

*Section 4: Food and non-food applications of Opuntia* spp.

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## Chapter 2

# South African Perspective on *Opuntia* spp.: Cultivation, Human and Livestock Food and Industrial Applications



Maryna de Wit  and Herman Fouché

**Abstract** The effect of scarce water resources, increasing desertification and climate change on food security prompted the exploration of the highly water-efficient, drought-tolerant cactus pear (*Opuntia ficus-indica* and *Opuntia robusta*) as a commercial crop for the food and other industries in South Africa. Cactus pear, formerly called the prickly pear, has long been valued in South Africa as cattle feed and for its delicious, healthy fruit. In 1914, 22 spineless Burbank *Opuntia ficus-indica* and *O. robusta* cactus pear cultivars were imported from the USA, and South Africa is the only country in the world where this collection is still found. Both *O. ficus-indica* and *O. robusta* are undervalued food sources, with the entire plant having value as a health-promoting crop with nutraceutical properties. Through research, the cactus pear can open up economic opportunities in South Africa. Research on cactus pears at the University of the Free State (UFS) is in collaboration with the South African Agricultural Research Council (ARC). The Waterkloof germplasm collection is located in the Bloemfontein district in the Free State, South Africa. A second germplasm was established on the west campus of the UFS in 2018 and a third site at Roodeplaats in Pretoria. All sites hosts 44 spineless Burbank cultivars, 42 of the *O. ficus-indica* spp. and two from the *O. robusta* spp. Research on human food application aspects as well as newly developed industries of this multi-functional crop is reported.

**Keywords** Cactus pears · South Africa · Feed · Food · Industries · Germplasm

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## Abbreviations

AFMA	South African Animal Feed Manufacturing Industry
AMMI	Additive Main effects and Multiplicative Interaction
ARC	Agricultural Research Council
Bc	Betacyanins
Bx	Betaxanthins
CAM	Crassulacean acid metabolism
CP	Crude protein
CSP	Chelating soluble pectin
CWM	Cell wall material
DM	Dry matter
FCP	Free choice profiling
FFA	Free fatty acids
GIT	Gastro intestinal tract
IV	Iodine value
m.a.s.l.	Meters above sea level
NMR	Nuclear magnetic resonance
NPN	Non protein nitrogen
ORAC	Oxidative radical
OSI	Oxidative Stability Index
PPWG	Prickly Pear Working Group
TA	Titratable acidity
TSS	Total soluble solids
UFS	University of the free state
WSP	Water soluble pectin
WUE	Water use efficiency

## 1 Introduction

The cactus pear (*Opuntia ficus-indica*) was introduced to South Africa (the Cape) over 300 years ago. It was carried thereafter, by settlers to arid and semi-arid parts of the sub-continent, during which the plants gradually reverted to its spiny forms over a period of approximately 150 years. Twenty-two spineless varieties of cactus pears (the so-called Burbank collection) were imported from California, USA to Middelburg in the Eastern Cape. Today the cactus pear is being promoted across South Africa as a versatile crop with multiple applications (UFS Webpage, [www.ufs.ac.za](http://www.ufs.ac.za), 2015).

Cactus pears, or prickly pears, are seeing renewed interest especially over the past 4 years from farmers in South Africa, due to climate change and drought as well as the plant's numerous uses. The drought conditions in many parts of South Africa help to boost interest of farmers to grow the cactus pears (Brown, 2020).

Cactus pear is generally classified as succulents and is part of the genus *Opuntia*, which includes about 160 species. Unfortunately, like any other family, the *Opuntia* family has its black sheep and includes the Jointed Cactus, Imbricate Cactus and a few others which pose a major threat to the environment. Luckily, not all *Opuntia* varieties are invaders, with the green-leafed *Opuntia ficus-indica* being one of the most common and popular edible varieties. The blue-leafed spineless cactus pear is known as *Opuntia robusta* (Fouché et al., 2020).

The Alien and Invasive Species Regulations of the National Environmental Management: Biodiversity Act defined the prickly pear as “an invasive species that must be destroyed”. Fortunately, farmers can legally propagate the spineless cactus pear (Brown, 2020).

The cactus pear is one of the most versatile crops available in South Africa, but is however, not receiving the needed attention. It has outgrown its limitation to only being used as drought-tolerant livestock feed and is currently producing orchards that yield food for human consumption, fruit, oil and animal feed. It is therefore crucial to give this miracle crop more well-earned attention. It is one of the most multi-purpose crops known, with no part of the plant going to waste (Brown, 2020).

The dedicated research and development programme at the University of the Free State (UFS) on spineless cactus pear (*Opuntia ficus-indica*)—also known as prickly pear—has grown steadily in both vision and dimension during the past years. Formal cactus pear research at the UFS started with the formation of the Prickly Pear Working Group (PPWG) in June 2002 and takes place with collaboration with the Agricultural Research Council (ARC) of South Africa (UFS Webpage, 2015).

The complexity of challenges to ensure sustainable food security and reduction of the impact of food production on the environment calls for more innovation in agriculture. The need to innovate emphasises the need for partnerships and joint ventures that will enable the role-players in agricultural value chains to ensure a sustainable agricultural sector. South Africa experiences significant socioeconomic challenges, including poverty, high unemployment rates, food insecurity and economic decline. These are compounded by adverse climatic factors. The effect of declining water resources, extended droughts, increased desertification and climate change on food security and economic growth prompted the South African government to develop policies and strategies that are targeted towards coordinated, multi-disciplinary,—institutional and—sectoral responses to critical national priorities. These priorities include job creation, skills and enterprise development, poverty alleviation, and food and nutrition security. Aligned to these strategies, the Agricultural Research Council (ARC) acknowledged the importance of strategic partnerships for the development of appropriate solutions (Venter et al., 2019). The objective was to establish a collaboration center on broadening the food base with the purpose of ensuring the availability of a wide range of nutritious crops. The methodology was to develop new/novel crops, food products and ingredients for enterprise development, value chains, income generation and job creation. The necessity for researchers to collaborate in addressing these complex problems is of overriding importance. By bringing expertise from various disciplines and

organisations together and leveraging other important networks and partners at both national and international levels, the benefits of greater returns within agricultural value chains will be realised. Cactus pear (*Opuntia ficus-indica* and *Opuntia robusta*) is increasingly becoming an important food, forage, cosmeceutical and nutraceutical crop in South Africa. It is a drought-tolerant crop used for a diverse range of applications. It was used mainly as a source of animal feed, but recent interest in its other potential uses saw the crop establish itself as an important multi-purpose crop, which opened up opportunities for the smallholder farming sector. Cactus pear was identified as a priority crop for research and development for the collaboration centre on broadening the food base. The purpose of this paper was to discuss the collaboration involved in developing this multi-functional crop within the context of the Sub-Saharan Africa Region Framework for Cactus Pear Research and Development. The establishment of the collaboration center provided a sound foundation on which further partnerships could be explored and developed. Valuable lessons were learnt concerning the maintenance of multi-disciplinary research teams and challenges for the development and establishment of new value chains for new crops in a country (Venter et al., 2019).

## 2 Background

### 2.1 *History (Introduction of Cactus Pear Plants to South Africa)*

The deportation of the Moors back to their homelands from Spain as well as the Spanish expansion (Ochoa and Barbera, 2017) opened avenues for cactus plantations in the African continent in countries such as Ethiopia, Morocco and South Africa. The Cape was the first area in South Africa where the cactus plant (*Opuntia ficus indica* species) inhabited. It was cultivated for the fruit as well as living fences in this area. During the coming 150 years, from the time they were first introduced, the cactus plants gradually returned to their spiny form (Ochoa and Barbera, 2017). This resulted in the infestation of impenetrable thickets in the Eastern Cape and other regions (Beinart and Wotshela, 2011). This cacti invasion was estimated to cover two million hectares in the early twentieth century in South Africa. This impacted the agricultural sector negatively. In an effort to combat the invasion, biological control was used to salvage approximately 80% of the infested plants (Zimmermann et al., 2009). These plants were cultivated mainly for fruit and animal forage.

Beside the plants that have been reported in South Africa as early as 1772, 22 spineless cultivars were imported by the Grootfontein Research Institute (Middelburg, Eastern Cape) in 1914 from the Burbank Nursery in the United States of America (AgriOrbit, 2019). These spineless cactus pear plants (*Opuntia ficus*



*indica* and *Opuntia robusta*) were established by Karoo farmers as livestock feed to mitigate the drought that was then present.

There are currently several germplasm banks which can be found in the Limpopo, Free State, Eastern and Western Cape provinces (Ochoa and Barbera, 2017). These secondary banks were established for research purposes which previously focused mainly on the use of the plant as forage (Menezes et al., 2010). With the increasing interest in the plant's application in human consumption and animal feed, particularly the young vegetables stems (nopalitos), the research focus has since shifted.

The Department of Agriculture in the Limpopo Province of South Africa has been conserving 78 accessions (including the Burbank varieties) in the largest spineless cactus pear germplasm bank in Africa at Mara Experimental Farm, Makhado (Potgieter and Mashope, 2009). The Mara Research Station is in the Vhembe district of the Limpopo Province. Mara ADC is located  $\pm 54$  km West of Makhado (previously known as Louis Trichardt), Limpopo Province at 23°05'S and 29°25'E, at altitude of 961 m above sea level in the Arid Sweet Bushveld. The average annual minimum and maximum temperatures recorded are 12.7 and 25.1 °C respectively. The average seasonal rainfall is 441 mm (Mokoboki et al., 2009). The Oudtshoorn germplasm (Western Cape) collection is located 314 m above sea level (m.a.s.l.) and receives on average 239 mm rainfall. This collection hosts 14 cactus pear varieties. The trial is laid out in a fully randomised design with two replications of each treatment. A second germplasm was established in the Eastern Cape at Cradock. This germplasm collection is located 846 m.a.s.l. and receives 359 mm rainfall on average. This orchard hosts 16 cactus pear varieties. The trial is laid out in a fully randomised design with two replications of each treatment. The first germplasm collection in Bloemfontein (Free State province) was established in 2005 by the ARC. This germplasm collection is located on the Waterkloof farm,  $\pm 20$  km west of Bloemfontein. It is located 1348 m above sea level (m.a.s.l.) and receives 556 mm rainfall on average. This site hosts 42 South African cactus pear varieties that are being evaluated for different uses. The trial is laid out in a fully randomised design with two replications of each treatment. There are 14 rows, each with six plots. Individual plots have five plants. The spacing is 5.3 m, adding up to the total plant population of 420 for the two replications (de Wit et al., 2010). Standard orchard practices as recommended by Potgieter (1997) are followed in all orchards. A second germplasm was duplicated and established in 2017 by the ARC and UFS on the west campus of the UFS main campus in Bloemfontein. At the same time, a germplasm collection was established at the Roodeplaat ARC experimental farm outside of Pretoria (Gauteng province). This site also hosts 42 spineless cactus pear cultivars (Fouché et al., 2020).

The cactus pear crop originated in Mexico and has a long and interesting history. Spanish explorers discovered the plants in Mexico in the 1700s and used the cladodes as vegetables on their ships. The fruit consequently were distributed all over the world. At around 1750, a South African farmer from the Graaff-Reinet district (Eastern Cape) brought two cladodes from the Cape and planted them on his farm, while another farmer from Somerset East, also cultivated cactus pears and used the cladodes to distill alcohol in the Kamdebo district. It was then used so commonly,

that during the early 1860s, farmers started to complain that cactus pears invaded the land and interfered with their sheep farming. The infestation escalated to such a degree that Henry Bolus described cactus pear, in 1874, as the biggest pest between the Suurberg and Sneeuberg mountain ranges (Beinart and Wotshela, 2011).

This infestation with prickly pear was aided by their spines that were too long, making it impossible for the livestock to graze on it. As part of the control measures, two insects, Cochineal (*Dactylopius opuntiae*) (1913) and Cactoblastis (cactus moth *Cactoblastis cactorium*) (1924) were imported for biological control at great costs. The popularity of this miracle plant was declining sharply (Beinart and Wotshela, 2011).

In 1914, the so-called spineless cactus pear, developed by Luther Burbank from the Burbank nursery in California, USA, was imported and planted by the Department of Agriculture at the research station Grootfontein, which is situated in Middelburg in the Cape province. In 1925, a paper that praised this new cactus pear cultivar as a valuable drought-resistant livestock feed was published (Turpin and Smuts, 1925). During this time, 22 cultivars of cactus pear were evaluated and today, this unique collection in South Africa is one of a few collections world-wide where these cultivars can be found. At first, the research focused mainly on the use of cactus pears for animal feed and, more specifically, nutrition during drought. The Cochineal-resistant blue-leafed spineless cactus (*Opuntia robusta*) was planted extensively in the Karoo and the cladodes fed to livestock. However, more recent agronomic evaluations established that cultivars with a much higher feed- and fruit production potential are available (Fouché et al., 2020).

The cactus pear fruit's potential were evaluated intensively for fruit production by the Department of Agriculture during the 1990s in the Limpopo and Gauteng provinces, and this period can be regarded as the second heyday of crop's history in South Africa. Renewed research into other uses of the crop also emerged during this period. The Cactus Pear Growers' Association and the Cactus Network (a group of interested researchers) were founded, and this resulted in an unprecedented interest in the crop. Currently at various locations in South Africa, several cultivars are being evaluated for their adaptability and production capacity. A research programme in the Free State has been initiated by the Agricultural Research Council (ARC) in 2001, where the cactus pear's potential as a feed source, among other things, is being investigated under semi-intensive conditions. Concurrently, several researchers at the University of the Free State had started research projects, investigating the plant's water consumption, its use as livestock feed, the fruit's quality and other human food applications (UFS Webpage, 2015).

## 2.2 Legal Regulations

In South Africa, some confusion as to whether cactus pear cultivation is allowed and regarding its status as an invader plant still exist. According to the Conservation of Agricultural Resources Act (Act No. 43 of 1983), *Opuntia ficus-indica* (L.) Mill.

was declared as a category 1 weed, which implies that no plants are to be planted, established, maintained, propagated, sold or acquired. It is therefore important to note that all spineless cactus pear cultivars and selections are exempt from this regulation. The *Opuntia* spp. are regarded as weeds, namely those that were introduced into a country outside their native habitats and became naturalized (Brutsch and Zimmerman, 1995; Zimmermann, 2010). Spiny cactus pear has invaded about 800,000 ha in South Africa, mainly in the Eastern Cape, and had to be controlled during the twentieth century with biological control. An Act was created that applied specifically to the spiny form, prohibiting the uncontrolled diffusion of the plants (Barbera, 1995; Brutsch and Zimmerman, 1995; Zimmermann, 2010).

### 2.3 Cultivation

Cactus pear, as a commercial fruit crop, is cultivated in at least 18 countries on more than 100,000 ha globally. These figures do not include cultivations for domestic use, small-scale farming or naturalized stands (e.g. as is found in the Eastern Cape Province). In SA, approximately 3000 ha have been planted exclusively for fruit, which support both domestic and export markets (Claassens and Wessels, 1997). More than 900 local farms devote a total of about 4500 ha to cactus pear production, including 1500 ha for fruit harvesting and 3000 ha for fodder production (de Waal et al., 2007). Cactus pear production is very labour-intensive especially during fruit harvesting. A farm needs about five people per hectare to harvest the fruit while during out-of-season, one worker per hectare is required to maintain the orchard. A hectare planted with cactus pear can yield between 20 and 30 tonnes of fruit per year. Assuming an average fruit yield of 20 tonnes per hectare, the value of the cactus pear fruit production harvested from 1500 ha at a price of R13333 per tonne could be worth at least R400 million (Brown, 2020).

## 3 Cactus Pear for Animal Feed

Water resources in South Africa are limited and above that, scarce water is increasingly prioritized for the human population. In view of the negative effects of climate change, crops that are more efficient users of water, especially in dry regions, are needed (AgriOrbit, 2019). Increasing demands on already scarce water resources in South Africa require alternative sources of animal feed. One alternative with the potential for widespread production is spineless cactus pear. It is 1.14 times more efficient in its water use than Old man saltbush, 2.8 times more efficient than wheat, 3.75 times more efficient than lucerne and 7.5 times more efficient than rangeland vegetation. Until recently, research has focused extensively on the use of cactus pear as drought fodder. However, this is now beginning to shift, with growing interest in the intensive production of spineless cactus pear for other types of animal feed.

Studies on the use of sun-dried cactus pear cladodes suggest that it has the potential to provide some 25% of the basic feed resources required by South Africa's commercial ruminant feed manufacturing sector (de Waal et al., 2015). Another example is the cactus pear fruit, produced seasonal, yielding large quantities of fruit in a relatively short period of a few months in summer. The fruit cannot be stored for a long period unless kept in cold storage. A procedure was therefore developed to combine large volumes of mashed cactus pear fruit pulp with dry hay and straw to preserve it for longer periods as high moisture livestock feed, called *kuilmoes*—a high water content livestock feed similar to silage (de Waal et al., 2015).

Production of high quality spineless cactus pear fruit is stimulated by pruning and this creates the opportunity to utilize cladodes as livestock feed. However, the high water content of fresh cladodes creates logistical challenges (De Waal et al., 2007), because large volumes of cladodes must be processed and dried for easier transport to where it is needed as livestock feed. Practical methods are thus needed to process and dry cladodes which will enable farmers with smaller orchards to store the dried cladodes for longer periods as livestock feed. Cactus pear fruit is produced seasonally, yielding large quantities of fruit in a relatively short period of about 2 months in summer, namely from about January to March (De Waal et al. 2015). Not all the fruit is suitable for human consumption, thus creating an opportunity to utilise another component of spineless cactus pear as livestock feed. Sun-dried cladodes and mashed fruit preserved on straw and hay can be used by ruminants, provided it can compete with conventional feeds.

Cactus pear, specifically the spineless form, is under-utilized as a multi-purpose crop in South Africa and this situation needs to be addressed urgently. Ruminant livestock utilizes veld (natural pasture or rangeland) as well as planted pastures as major feed sources. In South Africa, veld comprises about 80% of the land available for agriculture (De Waal, 1990). Veld is grazed by ruminants, such as domesticated cattle, sheep and goats, as well as indigenous wildlife. It is also utilised by other herbivore species such as domesticated equines. In parts of South Africa, planted pastures are produced under rain-fed conditions or irrigation and it is grazed by ruminants. Veld and planted pastures (the primary feed sources for ruminants) are augmented by feeds produced by the South African Animal Feed Manufacturing Industry (AFMA, 2014). According to AFMA, 11,380,587 tons of animal feed was produced in 2013/2014, including 2,057,788 tons for dairy cattle and 3,297,788 tons for beef cattle and sheep (AFMA, 2014). Most manufactured feeds are used to finish large numbers of cattle and sheep in feedlots but some of the feeds for beef cattle and sheep are used to supplement ruminants on veld and planted pastures. Cattle and sheep consume a variety of diets in feedlots, but in fact only require four basic feed categories in the diets. For ruminants, good quality roughage such as hay (grasses or legumes) and silage form the basis of diets. Second to that, animal performance is improved by increasing the digestible energy content of diets, with the inclusion of high energy feeds such as grain and grain by-products (the latter being derived from the human food industry). Thirdly, to achieve the high growth rates required by young ruminants, the crude protein (CP) content of diets are balanced by including high protein feeds such as oilcakes (plant origin), non-protein nitrogen

(NPN) and distillers by-products (the latter contains protein of microbial origin). The fourth feed category required to balance ruminant diets, is minerals and additives.

Spineless cactus pear fits the requirements, although some still regard it as a non-conventional feed resource (Negesse et al., 2009). In reality, spineless cactus pear is an under-utilized multi-use crop in South Africa. Spineless cactus pear, mainly *O. robusta*, was established in the Karoo by farmers as drought tolerant feed crops and the cladodes fed to livestock. Cultivars of *O. ficus-indica* have been promoted for export of the cactus pear fruit. In South Africa a distinction is made between green-leafed spineless cactus pear (*O. ficus-indica* and *O. fuscicaulis*) and blue-leafed spineless cactus pear (*O. robusta*) (Felker, 1995; De Kock, 2001). The famous blue-leafed spineless cultivars (Monterey and Robusta) produced disappointingly low yields, but should not be discarded as they are Cochineal resistant. In the past, the selection programmes mainly focused on fruit production and therefore the two cultivars had a low priority (Fouché et al., 2020).

As versatile as the plant is in terms of its fruit, the cladodes are just as valuable. Several options for utilizing the plant exist and these opportunities have not yet been fully exploited by the livestock industry in South Africa. Much can be learned from Brazilians and Mexicans, who have been using the crop for centuries, and where, in many cases, it is the only source of fodder. The main reasons why cactus pear is an ideal forage crop includes its: drought-resistance and production when other forage crops fail; wide adaptability to various climates and soils; production of higher yields than most pasture crops because of its high water-use efficiency; ability to be used and processed in various forms of feed; ease to establish and low establishment costs; suitability to use by small farmers and macro businesses alike; high quality feed yield; tastiness; and high digestibility (Fouché et al., 2020).

The plants are pruned to stimulate fruit quality and yield and in return, yield cladodes that can be utilised by livestock. Unfortunately, the high water content of cladodes restricts its use by livestock to the close proximity of orchards. Dry cladodes however, makes it is easier to process, transport and store material for longer periods. The University of the Free State is promoting sun-dried spineless cactus pear (*Opuntia ficus-indica* and *O. robusta*) cladodes (De Waal et al., 2015) and fruit (De Waal et al., 2015) as major ruminant feed sources. Despite the widespread use of cladodes as drought feed in South Africa, relatively little is known about the use in livestock production diets. In 2005 and 2008, increasing supplementation levels (0%, 12%, 24% and 36%) of sun-dried and coarsely ground cactus pear cladodes were included in balanced diets and was used to partially replace lucerne (*Medicago sativa*) hay in diets for young Dorper wethers. At the highest inclusion of sun-dried cactus pear (360 g/kg diet), there were no adverse effects on feed intake, digestibility, rumen variables, growth, animal performance and carcass quality. In fact, higher levels of dried cladodes in diets improved digestibility. The study further showed that the mucilage in cladodes is responsible for the occurrence of wet manure. Mucilage does not break down the digestive system and binds the water so that it cannot be absorbed from the intestinal tract (water is retained in the distal gastrointestinal tract (GIT)). Thus, the additional amount of water that sheep drank daily, as

a result of the intake of dried cladodes in the diets, was not excreted as expected by the kidneys as urine, but largely excreted in the form of wet manure (de Waal et al., 2015). The condition is sometimes confused with laxative or diarrhoea. Partially dried cladodes are increasingly fed to livestock by farmers. Recently, mashed cactus pear fruit was preserved on straw and hay as kuilmoes and it created a new use of spineless cactus pear as feed for ruminants.

Cactus pears are associated with the semi-arid zones of the world (Sáenz, 2000, 2002). It is tolerant to drought and an important characteristic of *Opuntia* spp. is the ability to produce large quantities of fresh cladodes, under relatively unfavourable conditions (Nobel, 1995; De Kock, 2001). Luther Burbank, pioneer of spineless cactus pear in the USA (De Waal et al., 2015), stated “The *Opuntias*, from root to tip, are practically all food and drink and are greatly relished by all herbivorous animals...” and, with specific reference to his breeding programme with the spineless cactus pear, concluded “The work is still in progress, but on a still larger scale and now these improved *Opuntias* promise to be one of the most important food-producers of this age ...” (Burbank, 1913 as cited in Fouché et al., 2020). *Opuntia* spp. are important livestock feeds in several countries (Felker, 1995; De Kock, 2001; Nefzaoui and Ben Salem, 2001), particularly during droughts and seasons of low feed availability (De Waal et al., 2007). The high water content of *O. ficus-indica*, about 100–150 g dry matter (DM)/kg also serves as water source for livestock in dry regions (De Kock, 2001; Nefzaoui, 2010; De Waal et al. 2012). Using cactus pear as livestock feed is a particularly attractive option because of its high water use efficiency (UWE) to produce DM (De Kock, 2001). Cladodes are used in several ways. Utilization by livestock with less waste is possible by chopping the cladodes (chaffing) before feeding. Chaffed cladodes can also be dried, milled and stored for later use during droughts or to feed fresh cladodes and increase DM intake (De Kock, 2001; De Waal et al., 2007).

It is not easy to enter the market as a viable option unless the feed has a competitive advantage (de Waal et al., 2015). Spineless cactus pear has a distinct advantage because it yields large quantities of DM as cladodes and fruit from less water compared to most feed crops. However, to compete effectively in the livestock feed industry with other feed sources the cladodes must be dried and processed and the fresh fruit must be preserved in a practical way as animal feed. Cactus pear is a good source of energy with a low crude protein content (2–3%), but with fertilization, it is well known that the crude protein content can increase to about 8%. Several studies have shown cactus pears as an important source of maintenance feed. In dry areas, the high water content is of great value, with approximately 90,000 l of water found in the cladodes produced per hectare (100 t DM/ha). On the other hand, this high water content impedes the intake of sufficient nutrients. Drying is hampered by mucilage, a water-soluble pectin-like polysaccharide. The well-known ability of cacti to retain water during adverse climatic conditions such as drought is due, at least in part, to the water-binding capacity of mucilage (Fouché et al., 2020).

### 3.1 *Various Ways to Use Cactus Pear as Feed*

#### 3.1.1 High-Density Plantings

Research from particularly Brazil has shown that crop yield can be drastically increased by increasing plant density. Intensive feed production goes along with intensive orchard management and practices. Dense plantings can be considered for feed production in higher rainfall areas or under drip irrigation. The utilization of cladodes can mainly be divided into two groups, namely to use the fresh plant material or dried. Both can then be processed further (Fouché et al., 2020).

Traditionally, fresh cladodes are well utilized by livestock either used whole, or cut into strips or cubes. The cladodes can be cut or shredded and partially dried before animals utilize it. Despite the form in which it is used, the high water content impedes the handling and processing of fresh cactus pear cladodes as well as its intake. Practical methods for shredding and drying cladodes can facilitate further processing. In the dry form, deficiencies in the nutrient composition of cladodes can be corrected during the formulation of balanced diets by adding supplements. Cladodes can also first be dried slightly in order to partially dispose of the high water content. The estimated cladode production of 100 tonnes of wet material per hectare (10 t DM/ha) with a plant density of 666 plants per hectare can be produced when orchards are pruned annually. Yields of up to 400 tonnes of wet cladodes/ha (40 t DM/ha) have been measured when the plants are planted exclusively as livestock feed under intensive conditions (Fouché et al., 2020).

#### 3.1.2 Shredding of Cladodes

Fresh cladodes can be shredded by multi-purpose shredders into thin strips to increase the surface area for drying in the sun. Thinner strips dry faster, although water from the slimy cladode pulp evaporates very slowly (nutrients are present in the juice) and the result is that the released mucilage solidifies on the surface and thus stops evaporation. The shredded strips are dried in direct sunlight on drying floors and on racks slightly raised from the ground to ensure free air circulation. Depending on the thickness of the strips, the weather conditions and further processing of the strips, the drying process can be completed within 4–14 days (Fouché et al., 2020). Thereafter, production diets can be formulated. Due to the interaction between mucilage and water, the role and relationship in the drying process of cladodes and especially in the digestive tract of ruminants are further investigated.

If fruit marketing is not the primary focus, the fruit part of the crop is available for livestock feed production. Results from various sources show that about 25–30 tonnes of fruit/ha are produced, and since some producers are only interested in seed production, the fruit pulp becomes a by-product and would therefore also be available as livestock feed. Ripe fruit contain about 12% of sugars (glucose and fructose), which make it a tasty, high-energy food source. Since the shelf life of the

harvested fruit is a few days, it complicates its use as feed, and must therefore be used or preserved as soon as possible. Some of the options are to make either silage or fodder blocks. Ripe fruit (preferably pulped) can be mixed with roughage, e.g. corn stalks or grass. Feed-grade urea and other micro-elements should then be added. The mixture is further ensiled in the standard way. The less air in the mixture, the better the silage quality. However, it is possible to make a good quality silage of the fruit (De Waal, 2015) without any additional molasses. In the agricultural industry, waste or by-products, such as olive cake, grape marc, citrus peels, tomato pulp and cactus pear fruit are obtained at production facilities. These products have a very short shelf life and must be used as soon as possible. A possible use of these waste or by-products is in the making of fodder blocks. This practice originated in North Africa, Arab countries and Iraq. In countries with few resources, this is seen as a major breakthrough in the animal nutrition industry (Nasri et al., 2008). The ratio in which the various elements are mixed is determined by the desired quality of the fodder block. Unused fruit or waste from sorting tables can be used as an energy source in the fodder blocks. Dried fodder blocks can be stored indefinitely and can therefore be used in times of drought. It can even be used as supplemental nutrition during winter and early spring. Silage can also be made from fresh cactus pear cladodes, but it requires a lot of practical experience. The silage is very wet and spoils easily. Furthermore, the quality depends on the sugar content of the roughage. If the cladodes are dried first, better results can be obtained. Shredded cladodes are mixed with dry roughage to reduce the water content.

Farming in South Africa is under increasing socio-economic pressure, often because of small and uneconomical farming units, increasing production costs, droughts and unreliable rainfall during critical periods of the growth season of natural pastures (veld). In the arid regions, South African farmers are predominantly keeping livestock or wildlife. They face seasonal losses during winter and early summer because of feed shortages, and managing risk has become part of their daily lives. South Africa urgently needs new initiatives for sustainable rural development. The scarcity of water resources created a need to find alternative sources of animal feed, specifically crops that use water more efficiently. The spineless cactus pear (*Opuntia ficus-indica*) is such a plant, and well adapted in dry environments, producing large yields of cladode and fruit for humans and animals. It has the potential to improve and stabilize the livelihoods of rural people. An initial group of 26 participants in the Oppermansgronde community have been assisted to plant 2-ha plots of spineless cactus pear under a rain-fed system. Spineless cactus pears have high water-use efficiency (WUE) and are adapted to marginal soils, and therefore do not compete with most cash crops. Cladodes are not a complete animal feed source, but they played a marked role in sustaining cattle and sheep during a number of consecutive very dry summers (2014–2016); the usual animal losses were limited and, on some farms, no losses occurred. Reproduction of sheep and cattle was affected, but less than expected. The data are still being evaluated, but an average household income of about US\$550 was generated from the sale of fruit and cladodes. Value adding and product development are also taking place in some households, with



produce being delivered to road stalls. This project created a working model for other community-based project (de Wit et al. 2019a, b, c, d).

## 4 Cactus Pear for Human Consumption

The spineless *Opuntia ficus-induca* and *Opuntia robusta* cactus pear species, imported to be used as fodder, necessitated its valuation for human food. Research on cactus pears for human food uses in SA was almost non-existent. It is a so-called “new crop” with immense potential for use in the food, nutraceutical, pharmaceutical and cosmetic industries. With a growing demand for nutritious food to combat food scarcity, the answer lies in under-utilized crops such as cactus pears. All possible applications of this exceptional drought-resistant CAM plant in human food should be evaluated. Research on cactus pears at the University of the Free State (UFS) is in collaboration with the South African Agricultural Research Council (ARC). Food Science research on cactus pears at the UFS started in 2006 and fruit was obtained from the Waterkloof experimental farm. The Waterkloof germplasm collection is located in the Bloemfontein district in the Free State, South Africa (GPS coordinates: 29°10′53″S 25°58′38″E). Climatic data are captured *via* an automatic weather station. The site is maintained under dry-land conditions, with rain as the only source of water. Two other experimental orchards developed and maintained by the South African Department of Agriculture are representative of different agro-ecological regions namely Cradock in the Eastern Cape (hosting 16 cultivars) and Oudtshoorn in the Southern Cape (hosting 14 cultivars) (de Wit et al., 2010).

### 4.1 Fruit Quality

One of the first research questions to be answered was if the fruit of all 42 cultivars being evaluated were suitable for human consumption, in other words, fruit quality was determined physico-chemically as well as sensorically. Data for Bloemfontein was compared to data from other secondary orchards established by the ARC and the Department of Agriculture at other agro-ecological regions namely Oudtshoorn (16 cultivars) and Cradock (14 cultivars). The influence of season and locality on internal cactus pear fruit quality parameters was established. These included fruit mass, pulp percentage, total soluble solids (TSS), pulp pH, pulp titratable acidity (TA), pulp ascorbic acid, pulp fructose and pulp glucose. Significant variation existed between mean values of the different characteristics (parameters) between localities (except for the pulp glucose values). Genotype X Environmental interactions were noted. It was concluded that Meyers is the most appropriate cultivar for economical purposes in South Africa (de Wit et al., 2010).

In the second study, the aim was to determine the influence of the location, cultivar and season on fruit quality, thus establishing the most stable cultivar for fruit quality characteristics among three agro-ecological localities (Bloemfontein, Cradock and Oudtshoorn). Fruit quality parameters were evaluated for 11 cultivars that were common to all three localities. The evaluation was done over two seasons (2008 and 2009). Parameters analyzed included fruit mass, pulp percentage, acidity, ascorbic acid- and sugar content. Significant differences in mean values among cultivars were found in the parameters tested at all the locations. Location contributed significantly to variation in sugar content, while cultivar contributed to variation in percentage pulp, pH and acidity. The interaction between location and cultivar however, contributed to variation in most of the parameters tested. The performance of the cultivars varied between seasons. Meyers was found to be the most stable at all three locations in the first season (2008), while Tormentosa performed the best in the second season (2009). According to the Additive Main effects and Multiplicative Interaction (AMMI) analysis, Meyers proved to be the most stable cultivar at all the locations over two seasons. Meyers also possessed substantial quality parameters compared to the other cultivars. Therefore it could be concluded that the genetic material, agriculture location and season made a significant contribution to variation in cactus pear fruit quality. The effect of location and season interactions was significant in especially sugar and acidic levels (Shongwe et al., 2013).

In a third study, the aim was to determine the fruit quality of cactus pear fruit from one locality (Bloemfontein) over two seasons. Physico-chemical quality attributes were evaluated for two agricultural seasons (2007 and 2008). The influence of rainfall and temperature on quality was determined. Highly significant differences were observed in terms of physicochemical composition among the 33 different cactus pear cultivars over the two seasons. This finding indicated that genetic differences among cultivars, as well as seasonal changes, have a significant influence on fruit quality. It was also evident from this study that not only the cultivar and agricultural season, but also the interaction between the cultivar and season had a significant influence on fruit quality (Rothman et al., 2013).

The fourth study provided results regarding the effect of cultivar and season on sensory quality of cactus pear fruit. Sensory quality was evaluated by Free Choice Profiling (FCP) over the two agricultural seasons of 2007 and 2008. The five most frequently-used attributes used by the sensory panel were sweet, sour, bitter, fruity and prickly pear, with the corresponding cultivars for season 2007 being Fresno, Robusta, Sharsheret, Malta and Amersfoort. For season 2008, the corresponding cultivars for the same attributes changed to Nudosa, Sharsheret, Robusta, Roly Poly and Ficus Indice, respectively. FCP could successfully distinguish between the two seasons, but not between the majorities of the cactus pear cultivars. The exception was observed in Monterey and Robusta, where FCP was able to differentiate these two cultivars clearly from the rest of the cultivars. In an attempt to determine if sensory quality of cactus pear fruit was influenced by the physicochemical parameters, Pearson correlation analysis was performed between the physicochemical and sensory data. Physicochemical attributes like pulp glucose, pulp fructose and percentage pulp were correlated with sensory attributes like sweet, fruity and prickly

pear. Positive correlations included “sweet” with pulp glucose and pulp fructose and “prickly pear” with pulp pH. Negative correlations were found between “prickly pear” and “sweet” as well as between “prickly pear” and % pulp (Rothman et al., 2012).

Consequently, in a fifth study, 12 cultivars with commercial fruit production potential in the Free State were selected from the Waterkloof orchard for the evaluation of fruit eating quality as well as the effect of climate (temperature, heat units and rainfall) thereon. These were evaluated over five production seasons. Rainfall had a significant positive effect on fruit mass, total soluble solids (TSS) content, glucose content, pulp pH and percentage titratable acidity (% TA), while rainfall had a negative effect on percentage pulp and betacyanin (Bc) and betaxanthin (Bx) pigment contents. Fruit mass, TSS, glucose, fructose content and % TA correlated negatively with total heat units, while % pulp, vitamin C content, pulp pH, Bc and Bx correlated positively with total heat units. Maximum temperatures had a similar effect on fruit quality to that observed for heat units, except for pulp pH. From these results, it could be concluded that all climatic factors measured correlated to changes in fruit quality, especially TSS, sugars, acidity and colour (pigments), although the effect of rainfall was most profound (Coetzer et al., 2019).

## 4.2 Processed Fruits

Processed fruits were also evaluated, e.g. cactus pear fruit juice including the effect of processing conditions (heat treatment, cooling and freezing) as well as further processing into fruit jellies. Cactus pear fruit juice was thermally processed (jelly manufacture included) and sensorially analyzed. Fruit from seven cactus pear cultivars used for human consumption and one, an animal feed cultivar, was peeled and the juice extracted. Three thermal treatments were applied namely freezing ( $-18\text{ }^{\circ}\text{C}$ ), refrigeration ( $4\text{ }^{\circ}\text{C}$ ), and pasteurization ( $60\text{ }^{\circ}\text{C}$ ). Ten semi-naïve panelists compared the taste by using their own descriptors and a ten point scale. Twenty-four descriptors were generated. The panel was successful in distinguishing between the cultivars used for human consumption and the animal feed cultivar. It was found that pasteurization had a detrimental effect on the flavor of the juice. Descriptive sensory analysis on cactus pear fruit jellies compared textural attributes such as cloudiness, smoothness, pectin content, runniness and cutting edge. Physical analysis of texture was also carried out in support to the sensory data. Only one significant difference between the seven cultivars for the sensory descriptor of cloudiness was found. Both physical tests (line-spread test and Brookfield viscometer) differed significantly between jellies from the seven cultivars (de Wit et al., 2014).

In a second study, a health drink was developed by combining fruit pulp and different teas. Ingredients such as tea, ginger, cinnamon, lemon juice, stevia sweetener, coconut water, and Ginkgo biloba were used alongside the cactus pear juice as functional ingredients in the development of an ice tea health beverage. The sensory panel consisted of 50 people and a 9-point hedonic scale was used to determine

consumer acceptance of the cactus pear ice tea. Analysis of variance (ANOVA) was used to determine the differences between the appearance, aroma, taste, mouthfeel, and aftertaste of the five different health drink formulas. Taste, aftertaste and aroma scored the lowest on the 9-point hedonic scale, while appearance and mouthfeel scored the highest on the hedonic scale. The results indicated that the formulas most liked were the Rooibos tea formula and honey-bush tea formula while the least liked formula was the green tea. The other in-between formulas included chamomile and regular black tea (Louw, 2018).

Alternative food uses of cactus pears: to establish cactus pears as a multi-use crop, other parts of the plant such as the cladodes and waste products should be investigated. These included fresh and processed cladodes as well as seeds and peels. Further exploitation and exploration on all human food applications of cactus pears, especially by-products and waste products of the whole plant should be done. Results obtained will broaden the application of this plant in arid and semi-arid countries where food sustainability is needed. With a growing demand for nutritious food to combat food scarcity, the answer lies in under-utilized, multi-use crops.

### 4.3 *Seed Oils*

A comprehensive study was launched into the oil composition and fatty acid analysis of the seeds from the cactus pear fruit. This oil is currently one of the most expensive oils to be used in culinary and cosmetic industries. Seed oils from the 42 cultivars from Bloemfontein (Waterkloof), 16 cultivars from Cradock and 14 cultivars from Oudtshoorn were chemically extracted, evaluated and compared over two seasons. Oil quality and stability was also determined. The total lipid content and fatty acid composition depends on various factors, including cultivar, degree of maturity, climate, harvesting season as well as agricultural practices. The first aim of the study was to conduct a seed oil quality analysis by investigating variation among the 12 common cultivars across the three locations (Bloemfontein, Cradock and Oudtshoorn) over two production seasons (2010 and 2011). Seed oil content and fatty acid content, as well as the ratios thereof, differed among cultivars, seasons and locations. Cultivar X location X season interactions were significant for oil content. Levels of oleic acid (C18:1c9) were significantly influenced by the cultivar X location interaction. Mono-unsaturated fatty acid content was significantly influenced by cultivar X location and location X season interactions. Oil content was significantly correlated with levels of palmitic acid (C16:0), stearic acid (C18:0) and oleic acid. The cultivars Van As, Turpin, Roedtan and Meyers showed good associations with oil content and C18:1c9, while Bloemfontein was the most stable location (Shongwe, 2012; de Wit et al., 2016).

The second aim was to evaluate the 42 cultivars from the Waterkloof orchard (Bloemfontein) over two seasons (2010 and 2011) for seed oil content and fatty acid composition in an attempt to determine the best oil yielding cultivar. Seed oil content varied among cultivars and seasons. The unsaturated fatty acids were the most

prominent and consist mainly of linoleic acid, followed by oleic acid the saturated fatty acids palmitic and stearic acid. Cultivar and variation in especially rainfall had a statistically significant effect on cactus pear seed oil content and fatty acid composition. Principal Component Analysis showed that certain cultivars were exclusively associated with specific characteristics. American Giant cultivar had the highest seed oil yield (Shongwe, 2012; de Wit et al., 2018).

The third aim was to evaluate and compare preliminary seven South African cactus pear varieties of *Opuntia ficus indica* and *O. robusta* for chemically extracted seed oil content, fatty acid composition and oil quality. Oil quality parameters included refractive index, iodine value, peroxide value, free fatty acid content, *p*-anisidine value and oxidative stability index. These oils demonstrated a relatively low oxidative stability index. Oxidative stability showed significant correlations with the oil content, oleic acid, stearic acid and monounsaturated fatty acid contents as well as with physicochemical properties such as the peroxide value and the *p*-anisidine value (Shongwe, 2012; de Wit et al., 2017).

Consequently, a fourth study investigated cold-pressed seed oil from 12 commercially produced cactus pear cultivars from the Waterkloof experimental farm (Free State) by comparing oil yield, fatty acid composition, physicochemical properties, quality and stability. Large differences in oil content, fatty acid composition as well as physicochemical properties (IV, PV, RI, tocopherols, ORAC, %FFA, OSI and induction time) was observed. Highest oil content was found in American Giant. The important fatty acids detected were C16:0, C18:0, C18:1c9 and C18:2c9,12, with C18:2c9,12 being the dominating fatty acid. Quality parameters of the oils were strongly influenced by the oil content, fatty acid composition and physicochemical properties. Oil content, PV, %FFA, RI, IV, tocopherols, ORAC and *p*-anisidine value correlated negatively with the oxidative stability index (OSI). American Giant was identified as the important cultivar with good quality traits (oil content and oxidative stability) (de Wit et al., 2021).

In a fifth study, the effect of oxidation on the quality and shelf-life of cactus pear seed oil, obtained by cold-pressing, was studied. Chemical and physical methods were employed to determine changes or analyse oxidative stability at both 25 and 30 °C at different stages throughout a 12 month storage period. Tests used included peroxide value (PV), *p*-Anisidine value (*p*-AV), refraction index (RI), free fatty acids (FFA) and oxidative stability index (OSI). Predictions of the shelf-life of the oils to be stored at 25 and 30 °C were made. This predicted shelf-life was found to be less than the actual shelf-life as determined by the comprehensive 1 year shelf-life evaluation. Both oils were very stable and it was concluded that storage time had a more pronounced effect on oil quality than storage temperature (de Wit et al., 2020a, b).

Further investigation into seed oils is being done. As part of an initiative to promote cactus pears as a multi-functional crop, dual-purpose cultivars should be identified and its production increased. This study is aimed to increase the yield and quality of the seed oil of *Opuntia ficus-indica* cultivar Morado produced for its commercially viable green-coloured fruit in South Africa. The project encompasses a trial using nitrogen (N) fertilization from three N sources (LAN, Ammonium

Sulphate and Urea) and four N application rates (0, 60, 120 and 240 kg/ha). It was found that fruit production significantly increased with both N source and application rate, however, the N source did not influence fruit quality, but high N application rates influenced fruit quality parameters negatively. Oil content significantly increased with increased N fertilization rates. The main fatty acids composition (oleic, palmitic, *cis*-vaccenic and stearic acid) were also significantly influenced, the highest content fatty acid, linoleic acid however, was not significantly influenced. It can therefore be concluded that N fertilization promoted an increased oil content, but was detrimental to the linoleic acid content. This study therefore provided important information on cultivation practices aimed at increased oil production (Nkoi et al., 2019).

## 4.4 Functional Ingredients

Functional ingredients in cactus pears include the powerful antioxidant colourants betalains, vitamin C as well as the soluble fibre mucilage.

### 4.4.1 Mucilage

Mucilage is a hydrocolloid with interesting functional properties. A patent was registered in 2011 by the UFS (Du Toit and De Wit, 2011) on the extraction of mucilage from cactus pear cladodes. This patented procedure included slicing, microwave cooking, macerating, centrifuging at 8000 rpm for 15 min, and freeze-drying at  $-60^{\circ}\text{C}$  for 72 h. An extensive study into the extraction, characterization and application of mucilage in food products as functional ingredient was completed. Further characterization and application of mucilage as native liquid and dried powder in functional foods was also undertaken.

In the first study, 42 cultivars (*Opuntia ficus-indica* and *Opuntia robusta*) were evaluated in terms of cladode morphology (size, weight, volume, width, length, diameter, and surface area), cladode moisture and solid contents, mucilage yield and mucilage viscosity. Cultivars were categorized into five groups in terms of mucilage viscosity, namely low, medium-low, medium, medium-high and high. Correlations between cladode size and weight, mucilage yield and viscosity as well as cladode moisture content and mucilage viscosity were determined for each cultivar. No correlation was found between size and weight of cladodes and mucilage yield. Positive correlations were found between cladode moisture content and mucilage yield and mucilage viscosity, while the highest positive correlation was observed between the mucilage yield and mucilage viscosity. Cladode dimensions and properties were linked with mucilage yield in order to select cultivars for further analysis in food applications (de Wit et al., 2019a, b, c, d).

In a second study, the unique flow behavior of mucilage was determined. The flow behaviour should be fully understood in order to predict its behavior during

processing, packaging, preparation and consumption. Extraction by the patented procedure and drying of mucilage from mature cladodes from four cultivars, harvested in winter (July 2014) was done. The four cultivars had viscosities between 26.0 and 29.8 cm (not significantly different) using the line-spread test and 150 and 328 cP using the viscometer. In the controlled-rate and time-interval tests, mucilage showed non-Newtonian, pseudoplastic tendencies but no rheopectic or thixotropic behaviour. Mucilage exhibited dynamic yield, indicating the force needed for it to start moving. Viscosity increased at lower temperatures and decreased at higher temperatures. In alkaline regions (pH > 11 to 8), viscosities of native mucilage increased, while they decreased in acidic (pH 6 to 1) regions. Monovalent ions had little, divalent more, and trivalent the most influence on native mucilage viscosity (du Toit et al., 2019a, b, c, d).

In a third study, the investigation of the functional properties of extracted freeze-dried powders was determined to predict the potential of South African cultivars as healthy ingredients. Freeze-dried powders of four cultivars (*Opuntia ficus-indica* Algerian, Morado, Gymno-Carpo, and *Opuntia robusta* Robusta) were dissolved in water or oil. The solubility-, swelling power, water and oil holding- and absorption capacity as well as emulsification capacity of the mucilage powders were determined. Freeze-dried mucilage powders had a very high solubility index, water- and oil absorption as well as water- and oil holding capacities. It displayed high emulsification capacity and stability. The protein content for the three *O. ficus-indica* cultivars was not significantly different, but significantly higher in *O. robusta*. None of the cultivars possessed significantly better functional properties. Native mucilage was applied in fat-, dairy and egg-replacement products. Mucilage showed promise as emulsifier and fat-replacer in mayonnaise products and was described as creamy with a highly acceptable mouthfeel. It showed strong potential as a functional ingredient (du Toit et al., 2019a, b, c, d).

During a fourth study on mucilage, it was speculated on the variation in physico-chemical and technological properties of powders, when cultivar and harvest month of cladodes differ. This, in turn, could lead to differentiation in the application of mucilage powders. Three *Opuntia ficus-indica* (Algerian, Morado and Gymno-Carpo) cultivars and one *Opuntia robusta* (Robusta) cultivar were harvested over a 6 month period (February to August) and evaluated. February mucilage powders were the most porous with highest oil absorption and oil holding capacity, lowest water holding and swelling capacity, and lowest ability to increase viscosity. August mucilage powders had the smallest impermeable particles, highest water holding and hydrophobic properties, as well as the best emulsifying capacity, stability and ability to increase viscosity. *Opuntia robusta* produced brighter, darker green, more viscous mucilage while *Opuntia ficus-indica* powders were dull, light yellow-green with a lower viscosity and emulsifying capacity. Robusta mucilage was successfully applied in mayonnaise products as emulsifier and fat replacer (mimic) to replace up to 50% egg yolk and 30% oil (du Toit et al., 2019a, b, c, d).

The cultivar with the most optimal nutrient content and the preferred harvest times was still unknown. For that reason, in a fifth study, mucilage from three *Opuntia ficus-indica* (Algerian, Morado and Gymno-Carpo) and one *Opuntia*

*robusta* (Robusta) cultivar were investigated to determine their nutrient content over 6 months (February to August). Nutrients that contribute energy were low. The mineral (ash) content was high, particularly calcium and phosphorous. Low insoluble acid-detergent fibre and neutral-detergent fibre values indicated that mucilage was mostly soluble fibre. Calcium oxalate crystals were not detected in dried mucilage. *Opuntia robusta* powders had higher protein, extractable fat and potassium content, while *Opuntia ficus-indica* mucilage powders had higher polyunsaturated (linoleic and  $\alpha$ -linolenic acid) fat content. *O. robusta* Robusta mucilage, harvested after the fruit harvest (February) had the lowest energy content and the highest mineral and protein content (du Toit et al., 2018).

During a sixth project, mucilage as gellant in marshmallows was investigated. The objective of the study was to replace gelatin in marshmallows with different concentrations of fluid (native) mucilage, combined with different concentrations of powdered hydrocolloids. Nine different formulations were prepared with combinations of mucilage, xanthan gum, guar gum and agar-agar. Consistency, texture, tenderness of gel and shear measurements were determined, along with color ( $L^*$  values, as well as  $C^*$  and  $H^\circ$  values) and  $a_w$ . Significant differences between the different samples for all measurements were found. The best formulation for gelatin replacement was found to be the 75% mucilage +12.5% xanthan +12.5% agar combination, as it only differed significantly from the control (100% gelatin) sample in regard to shear. It was significantly less tender and resembled the shear of commercially available marshmallows in South Africa. All samples had a light, greyish yellow color (du Toit et al., 2016a). In the subsequent part of this study, the aim was to compare consumer liking of flavored and unflavored marshmallows made with native liquid mucilage, to that of a flavored and unflavored control sample (with 100% gelatin), as well as a flavored and unflavored commercial brand (Manhattan). Consumer liking was tested for taste, aftertaste, texture, as well as an overall acceptability of liking by a panel of 92 consumers. The white mucilage marshmallows had the lowest ranking for taste, aftertaste, texture and overall acceptability, and differed significantly from all the other samples. However, the pink mucilage marshmallow did not differ from the pink commercial one (which had the highest rankings for taste, aftertaste, texture, and overall acceptability) and pink control marshmallow. It could be concluded that flavoring successfully masked the distinctive aroma of the mucilage in the marshmallows, thereby also increasing scores for texture and overall acceptability (du Toit et al., 2016b).

### Inclusion of Mucilage as Functional Ingredient in Food Products

Functional food products are not medical food (specific formulations used for specific disorders or diseases used under medical supervision), nor are they dietary supplements. They are products that already form part of normal dietary patterns but address special dietary requirements. Mucilage in its native and freeze-dried form is an ideal candidate for such innovative functional food products, as it has been proven to enhance the nutritional content of food, while replacing undesired and



unhealthy ingredients. The aim of the first application study was to evaluate the sensory acceptability of firstly using mucilage as stabilizer and fat-mimic in ice-cream and sorbet, as well as to evaluate the effect of cold processing and frozen storage on the functionality of mucilage, while secondly to use mucilage in the manufacture of yoghurt, to increase soluble fiber content as well as its contribution to water-holding capacity and stability. Native liquid mucilage was used in altered formulas in order to make dairy-free sorbet as well as fat-free ice-cream using skimmed milk. Freeze-dried mucilage was added in different concentrations to plain, unflavored yoghurt. A naïve consumer panel of 50 people was used to for sensory analysis using preference ranking tests to indicate the acceptability and preference of the products using 9-point hedonic scale tests. Mucilage had an adverse effect on the ability of the ice-cream and sorbet to freeze. Large ice crystals were observed in the mucilage replacement products. All the attributes of the replacement products were significantly lower than the control ice-cream and sorbet. The increased addition of mucilage improved the consistency as well as the sensory properties (attributes) of the yoghurt. Therefore mucilage addition had an adverse effect on crystallization of ice-cream and sorbet, but could be used as fiber additive in yoghurt (de Wit et al., 2019a, b, c, d).

The second application study attempted to apply mucilage as gelling and emulsifying agent in edible films and spherification (edible beads). Since mucilage is a hydrocolloid, a gelatinous slimy substance that contains polysaccharides and proteins, and its ability to absorb huge amounts of water which result in modified viscosity, it can be used as a food additive in the food industry as a hydrocolloid to modify food texture. It is used as emulsifier, to form gels and edible coatings. It is a good alternative to the current hydrocolloids and synthetic coatings because of its availability, chemical free production, low calories and cost. Mucilage was extracted by the patented method from four cultivars, namely *Opuntia ficus-indica* and Robusta (*Opuntia robusta*). It was then dried in two ways, freeze-drying and hot-air dehydration. Emulsifying capacity and gelling capacity was tested. Edible coatings were made by mixing mucilage, water and glycerol. For spherification, mucilage was used to replace the usual gelling agents. Mucilage showed good ability to form stable emulsions. During gelling ability tests, mucilage from Robusta showed greater ability to gel, compared to the other three cultivars, but it was concluded that mucilage does not form true gels on its own, but rather improve gel formation with other hydrocolloids. It can form edible coatings which slow down colour, weight and firmness loss in vegetable and fruits. It also acts as an anti-browning agent in potato chips, reduces oil absorption during frying and enhances colour during frying. When mucilage is used on its own to replace sodium alginate in spherification, it does not gellate nor formed the spheres, but it formed a gel membrane when incorporated with xanthan and agar. Mucilage can be a replacement to food hydrocolloids. It has the ability to stabilize emulsions and form edible coatings. Although it cannot form a true gel, when incorporated with xanthan and agar it forms a strong gel (Mushanganyisi et al., 2019).

## Mucilage Protein Fraction Characterization

The protein fraction of the mucilage, being responsible for its emulsification and stabilization properties, must especially be characterized. In 1982, Trachtenberg and Mayer found low molecular weight substances in their extracted mucilage, but mistake it for contamination debris. Majdoub et al. (2001) found 2.2% proteins to exist in mucilage by ultra-filtration techniques. In 2012, Gebresamuel and Gebre-Mariam found protein content to be 6.82%. Du Toit et al. (2018) found 2.52–5.74% protein in mucilage. The aim of this study was firstly to determine and compare the protein fraction content of the mucilage extracted by the patented method from 40 *O. ficus-indica* and two *O. robusta* cactus pear cultivars from the UFS west campus collection. The second aim was to determine its foaming capacity and stability in foam food systems. The third aim was to quantify and characterize the proteins and their amino acids from mucilage to understand their effectiveness in reducing surface tension in foams in comparison to that of well-known and used foaming agents in the food industry. The LECO thermal combustion N and protein determination delivered a higher protein concentration in all the cultivars compared to the Bradford method's results. Protein concentration of crude mucilage was also significantly lower than that of Albumin and Sodium caseinate. Five cultivars namely Robusta, Malta, Gymno Carpo and Algerian, with the highest foaming capacity and stability were selected for further fractionation and characterization. SDS-PAGE showed that Robusta was the only cultivar that showed no bands and had a low protein content, although it showed foam stability properties. This could be due to the sugar content. The common sugar in all cultivars was glucuronic acid. Gymno-Carpo contained only glucuronic acid and xylose (Miya et al., 2019).

## Mucilage from Fruit Peels

An attempt was made to extract and characterize the saccharides from cactus pear peels. Six cultivars were used, namely Nudosa, Turpin, Ficus Indice, Muscatel, Skinners Court and Vryheid—all from the *O. ficus-indica* spp. These were selected based on earlier fruit quality and genetic analysis (Mashope, 2009; De Wit et al., 2010). The extraction and fractionation method of Habibi et al. (2004a, b) was used. The fat and wax to cell wall material (CWM) ratio was more or less the same for the cultivars. The mucilage content varied between the cultivars with Ficus Indice having the highest percentage of mucilage (~2%). The water soluble pectin (WSP) content was found to be constant among cultivars, while the chelating soluble pectin (CSP) varied considerably. Mono-, di- and polisaccharides were analysed by 1H-NMR analysis. All the cultivars had very similar results. The mucilage structure of the cultivars was very similar to each other. A comparison between the mucilage structure and pectin structure indicated two distinguished polysaccharides (results not published).

#### 4.4.2 Cladode Flour

##### Baked Products

The influence of cladode flour addition to different baked products was investigated. This was done to increase fibre content and replace wheat flour (gluten). Cactus pear (*Opuntia ficus-indica* and *O. robusta*) cladode flour was used to prepare and evaluate three types of baked products. In a health bread, whole wheat flour was replaced with cactus pear flour in percentages of 2%, 4%, 6%, 8%, 10%, 12%, and 17% replacement. The volume decreased and the texture became more solid and firm. The brown colour darkened when the percentage replacement of the flour increased, although it was still acceptable for the consumer. Oats crunchy biscuits were made with increasing replacement levels (0%, 5%, 10%, 20%, and 50%) of wheat flour with cactus cladode flour from three different cultivars, *Opuntia ficus-indica* (Skinners Court and Morado) as well as *Opuntia robusta* (Monterey). Cultivar had a significant effect on colour, taste and texture, but not on appearance. Increasing inclusion levels of cactus pear flour had a significant effect on all the evaluated sensory attributes. The taste most liked by the panel was that of the Morado 10% inclusion level sample. Cladode flours affected quality parameters of texture, colour and taste of the biscuits. With the increase in the level of cladode flour in the formulation of a popular South African carrot cake, the sensory scores for the organoleptic characteristics of the cakes decreased. From the overall acceptability rating, it was concluded that cladodes flour could be incorporated up to 25% level in the formulation of cakes (Van den Berg, 2012; de Wit et al., 2015).

##### Traditional, Indigenous Fermented Beverages

This study focused on the fermenting properties of *O. ficus-indica* cladode flour in traditional, indigenous beverages mageu and beer. The main fermenting agent involved in both products was maize meal. In the case of the beer, King Korn (sorghum/millet) was the key ingredient. Mageu is a traditional fermented drink made from cooked maize meal, that is left to sour over a period of 5 days. The pH of all samples tested had a lower pH after fermentation. The presence of higher levels of lactic acid bacteria (after fermentation) in all the samples indicated that sugar had metabolized to lactic acid, leading to a lowering in the pH. As the levels of cactus meal inclusions increased, the colour of the samples got darker in comparison to the control. The best sample contained 20% cactus meal inclusion. The sample showed all the characteristics associated with the control sample of the mageu. Sensory analyses of the control and 20% inclusion were done. Analyses found that the panel of consumers did indeed notice a difference between the two samples. The panel had favored the control mageu over the cactus mageu (Van der Bijl, 2013).

A traditional, indigenous beer, locally also known as “platpit” beer was made from fermented maize meal and sorghum. The fermenting process normally takes up to 5 days. The sorghum (King Korn in this study) is the fermenting agent. What

was evident through this study is the relationship between pH, yeast, lactic acid bacteria and alcohol formation. As was the case with the mageu, the pH levels of all beer samples were more acidic after fermentation. The sample with a 25% cactus meal inclusion was the best. This sample had all the characteristics that were found in the control beer. A sensory analysis was also conducted on the beer, and two samples were used, namely the control and the 25% inclusion. The panel had favoured the cactus beer over the control beer. It was clear that the cactus meal could be used in the making of traditional beer at an inclusion level of 25%. It might be able to use in higher inclusion levels as well, with the addition of certain food additives (Van der Bijl, 2013).

#### 4.4.3 Antioxidants

Antioxidant content and potential of cactus pear fruit (pulp, peel and seeds) and cladodes from fresh and processed products were evaluated. In the first study, the fresh fruit (pulp), peel, seeds and cladodes of each cultivar were compared. Analysis included betalains, ascorbic acid, phenolics and carotenoids. The activity of the antioxidants was determined by using the DPPH method and by measuring the chelating activity of ferrous ions. When % DPPH was tested, peel and cladodes were consistently the highest, while in the % chelating activity tests, fruit pulp and seeds were the best tissue types. Cladodes contained more phenolics and carotenes than fruit regardless of the cultivar. For pulp and peel, the cultivar that contained the highest antioxidant content and potential was Robusta (purple) with its high content of betalains followed by Gymno-Carpo and Ofer (both orange) with high ascorbic acid levels. The study proves that the fruit (pulp), peel and seeds from different cultivars contain specific antioxidants relating to the colour of the fruit, but the cladodes of any cultivar contain similar and highly effective antioxidants (du Toit, 2013; de Wit et al., 2019a, b, c, d).

The aim of the second study was therefore to explore the processing of cladodes into different preserved food products and to study the processed products' antioxidant content and capacity compared to the fresh cladodes. All cultivars demonstrated high antioxidant capacities and therefore, the cladodes of any cultivar could be considered as suitable for processing or preserved to produce healthy products. Processing had a greater influence on the antioxidant content of cactus cladode products than the cultivar. Dried products were the best in terms of antioxidant content and capacity (du Toit et al., 2018b).

During the third study, cactus pear fruit pulp was investigated. The aim of the study was to determine the relationship of fruit processing method and colour with antioxidant content and activity in fresh and processed cactus pear fruit. Antioxidant components (ascorbic acid, phenolic compounds, carotenes and betalains) and antioxidant activity (radical scavenging activity towards DPPH and Fe-chelating capacity) were determined in fresh and processed (juiced, dried, preserved and chutney) fruits from four different colored cultivars (purple, green, orange and pink). The highest antioxidant content and potential was found in purple (*O. robusta*

cv Robusta) fruit products, attributed to the highest levels of betalains (1140.4 mg kg<sup>-1</sup>). Orange fruit (*O. ficus-indica*) products had the second highest levels, attributed to ascorbic acid and phenolics. Betalains were highly retained in all processed products, while ascorbic acid was mostly retained in the processed products that involved minimal heat treatment. Carotene and phenolic compounds became more available for extraction during processing and showed higher levels after processing. Principal component analysis makes it possible to identify fruit colours of fresh and processed products, which were mostly associated with a specific antioxidant. PCA indicated that fresh purple fruit was correlated with chelating activity and betalains, while orange fruit was correlated with phenolics, ascorbic acid, carotene and DPPH. For the processed fruit products, most were clustered together with chelating activity, DPPH and the antioxidants. Orange and pink dried products had high ascorbic acid, phenolics, carotene and DPPH values, while dried and fresh purple fruit had high betalain content and chelating activity (du Toit et al., 2018a).

In a fourth study the antioxidant content and antioxidant potential of fresh and processed (juiced, dried, preserved and chutney) cactus pear fruit peels from different fruit-colored cactus pear cultivars were investigated. Cactus pear peels contained high levels of antioxidants and demonstrated high antioxidant activity. The highest contents were found in dried peels, while the preserves had the lowest contents. PCA analysis indicated that products, rather than cultivars, seem to cluster together. Robusta and its products cluster together, as well as with betalains. The % DPPH, carotenoids and phenolics are grouped together, with % chelating activity closely correlated with ascorbic acid. Dried products from all cultivars correlated closely with % DPPH, carotenoids and phenolics, especially dried peel from Gymno-Carpo (orange), Ofer (orange), Meyers (red) and Nepgen (green). Purple fruit peel products had the highest % DPPH, % chelating activity, betalains, phenolic compounds and carotenes. Ascorbic acid dominated in orange and red fruit peels. Purple and orange were the colours of cactus pear fruit cultivars that might be the best choice in terms of antioxidant content. The cultivar that presented the best fruit peel from an antioxidant point of view for preservation was Robusta. Cactus pear fruit peels should be included in processed products such as juice, dried fruit and chutneys (de Wit et al., 2020a, b).

#### 4.4.4 Colorants

A study was done on the extraction, characterization and application of betalains from different colored cactus pear fruit compared to that of beetroot and purple amaranth leaves. A natural and stable powdered colorant was extracted, dried and applied in food products. The shelf-life of these colored products was determined. Natural colorants promote health safety and are often preferred by consumers. As such, the use of betalains, which are naturally derived pigments with nutritional benefits such as vitamin C and antioxidants that are vital to the end-user was explored. To ensure maximum betalain extraction at low cost, different extraction

methods were investigated. Colorants were extracted from red beetroot, amaranth and eight cactus pear cultivars from the following colours: green, orange, red/pink and purple. The cultivars included: American Giant, Morado (both green), Ficus Indice, Gymno Carpo (both orange), Algerian, Meyers (both pink-red) and Monterey, Robusta (both purple and from the *O. robusta* spp.). Results showed that the best extraction solvent in the study was distilled water. Green cactus pear cultivars contained the lowest amount of betalain, while the highest concentration was found in the purple cultivars. The best extraction temperature was 25 °C, and most betalains remained stable until 80 °C (Sigwela et al., 2018). Natural colorant guidelines from the European Commission further revealed that the food definitive safety patterns followed during the extraction phase of these colorants allow them to be labelled as Colouring Food. They are labelled as fruit or vegetable juice with inherent coloring abilities and acceptable for use in countries around the globe. Betalains transmitted color and subsequently remained stable under various production methods. They successfully colored various products including baked, pan-fried, emulsified-cooked meats, jellies, candies and dairy products (Sigwela, 2020).

#### 4.4.5 Seed Proteins

Studies have been done on the extraction and characterization of seed proteins from the cactus pear seeds. The aim of the first investigation was to determine the content and to characterize the different seed proteins of three cactus pear cultivars (*O. ficus-indica* Algerian and Meyers as well as *O. robusta* Robusta) from three different locations (Bloemfontein, Cradock and Oudtshoorn). Results indicated that location and cultivar had no effect on the total nitrogen and total protein content. Significant differences in free amino acids levels between locations were observed. Urea and SDS polyacrylamide gelelectrophoresis (PAGE) analysis of the proteins revealed the following fractions: a 15 kDa protein band (in the 2S Albumin group), three protein bands within the Prolamins group (Mr range of 37, 50 and 75 kDa) as well as a 40 kDa protein band in the group of the 11S Globulins (Lebeko, 2010).

In a second investigation, three cultivars namely Morado, Meyers (both from *O. ficus-indica* spp.) and Robusta (from *O. robusta* spp.) from three locations (Bloemfontein, Oudtshoorn and Cradock) were compared over two seasons (2010 and 2011). Proteins were extracted with a step-wise ammonium sulfate (AS) precipitation procedure using different concentrations, namely 40%, 60% and 90%. A step-wise AS precipitation method, that increases the AS concentration, is better to use than just one extraction concentration. A de-fatted fraction was also included in all the analysis. The various cultivars differ from one another for each of the locations as well as the seasons. These differences included the colour of the fruit, the season and location, as well as the determined protein content and composition. De-fattening caused the fat-soluble proteins to be removed. De-fattening could also distort protein characterization, because proteins are selectively extracted. The protein concentration of the supernatants (both methods) for the 2010 season was marginally higher compared to the 2011 season. Both seasons showed expected

decreases in supernatant concentrations with increasing AS precipitation concentrations. The SDS gels showed similar results for the cultivars and locations and seasons, although different bands were observed for the different AS concentrations. The only distinct bands (except that of below 10 kDa) were from the 40% AS precipitation. Only 60% and 90% AS showed distinct bands below 10 kDa. A range of different sized bands between 23 and 100 kDa were analysed by MS. A variety of different proteins was found, from metabolic-type proteins to structural-type of proteins. The enzymes, for example Aspartate amino transferase and Putative aspartic protease, are metabolic proteins. The structural proteins included Caleosin. Caleosins are proteins involved in storage lipid mobilization during seed germination (Daffue, 2014).

#### 4.4.6 Cladodes (Nopalitos)

Nopalitos have been considered a valuable food source and enjoyed in cultural cuisines. The nopalito production has been thriving and sustaining people in most countries for many years. According to studies, it is identified as a vegetable with nutritious benefits and can be used for medicinal purposes.

The aim of the first project was to determine the morphological and physicochemical quality characteristics of nopalitos harvested from 20 cactus pear cultivars from 2 seasons (2018 and 2019) from the UFS west campus collection. Nopalitos from 20 cultivars were harvested and morphological and physicochemical tests were conducted including weight, moisture content, solids, sugar content, acidity, color, mucilage content and viscosity thereof, and pH which are some of the main factors which affect the palatability and overall functionality of the plant. Interesting trends were noticed between the two seasons with the 2018 harvest scoring lower than the 2019 harvest in all attributes. The acidity, pH and sugar content of the samples were generally higher in 2019 than in 2018. These attributes affect taste, keeping qualities and overall acceptability of the samples. Overall, 2019 season was better than 2018 with the best cultivars having been Meyers, Malta, Nudosa, Fuscicaulis, Fresno and Morado. The reported work has shown acceptable quality of the nopalitos as a vegetable source (Makhalemele et al., 2019).

In the second project, nutritional analysis was conducted for the six nopalito cultivars which were deemed to be the overall best, following the morphological and physicochemical analysis of 20 South African cactus pear cultivars (Makhalemele, 2020).

In a third project, the sensory profile, as well as the consumer acceptability of nopalitos from 20 South African cactus pear cultivars, as well as cucumber and green pepper (vegetable controls) were determined. Sixty-one consumers ranked the overall liking of the samples on a 9-point hedonic scale. The same panelists selected sensory characteristics, which they best associated with certain attributes, by using the Check-all-that-apply (CATA) question. The CATA contained 32 attributes, divided into six categories, namely color (green), appearance (fresh, thin, thick, slimy), taste (sweet, sour, salty, bitter, savory/umami), texture (stalky, chewy,

fibrous, slimy, hard, spongy, crisp, soft), aftertaste (sweet, sour, bitter, savory/umami, metallic, none) and flavor (grassy, cucumber, green pepper, green bean, fresh, mild, bland, herbal). No significant differences were noted between the hedonic scaling for the nopalito cultivars. There were significant differences observed for 25 of the CATA attributes, between the 20 cactus pear cultivars and two control vegetables. Among the 20 nopalito cultivars, Skinners court, Turpin, Fusicaulis and R1251 were ‘neither liked nor disliked’ by the consumers, as they were all ranked higher than the other cultivars. The remaining 16 nopalito cultivars were ‘disliked slightly’ by the consumers, with the least liked cultivar being Robusta (Makhalemele, 2020, see p. 32).

In the fourth project, two selected cultivars (Morado and Fusicaulis) were evaluated for the influence of harvest size (9, 12, 15, 18, 21, 24 cm), harvest season (post-fruit harvest; March and spring growth; September) and cultivar on the eating quality. A comparison to well-known vegetables (baby marrow, carrot, celery, cucumber, green beans, green pepper, onion, tomato) was undertaken. The preparation techniques and cooking methods were determined, and several recipes were developed. The recipes were evaluated by consumer panels consisting of ten members using 9-point hedonic scale tests. The panelists completed a questionnaire to reflect their attitudes as consumers. This study evaluated the quality characteristics in terms of morphology, colour, texture, turgidity, sliminess and gustatory properties of nopalitos. The quality characteristics observed in this study provided information on mostly consumed known vegetables comparable to *Opuntia* nopalitos. The two nopalito cultivars (Fusicaulis and Morado) showed good quality characteristics in terms of consumer preferences (Mpemba et al., 2019; du Toit et al., 2019a, b, c, d).

The use of fresh cladodes in a vegetable juice manufacture is also being evaluated. A consumer acceptance study was done to evaluate the acceptability of cactus cladode juice blends by consumers. The results obtained from this study indicated that the cladode juice blend was considerably well accepted. The guava juice blend had the highest scores of liking for attributes aroma, taste, mouthfeel and overall liking. A fruit juice/cladode blend, rather than a vegetable juice/cladode blend, should be considered, i.e. a guava/cladode juice blend, followed by a kiwi & pear/cladode juice blend (Muller, 2013).

## 5 Industrial Applications

### 5.1 Economic Benefits

The processing of cactus pear in South Africa is relatively limited. This implies that there are many opportunities to develop an agro-industry. In many plants suitable for human and animal consumption, only a few parts of the plant are utilized. On the other hand, cactus pear is a crop of which the fruit, cladodes and even the flowers



are useful. South African cactus pears, especially the red fruit varieties, are very popular in the European market, where it is sold as a delicacy. Less common uses of cactus pear include flower and cladode arrangements, jam and even soap. Even the cochineal insects can be used in the cosmetic industry in the manufacturing of lipstick and other make-up products. In many countries including South Africa, some countries in South America, as well as the Mediterranean (Mediterranean), only the fruit is eaten. In Mexico, the tender young leaves (nopalitos) are also eaten. However, both fruit and nopalitos are perishable and processing technology is needed to extend shelf life. Furthermore, both the fruit and cladodes contain many bioactive ingredients that must be preserved during processing. Cactus pear is a versatile fruit and a wide range of products and by-products can be obtained from it. The same goes for the cladodes. Research suggested that the economic benefits of cactus pear production could be important. A recent study demonstrated that young plants pruned to stimulate fruit quality yielded 8000 kg of cladode dry matter per hectare after only 4 years (Brown, 2020).

### 5.1.1 Developing Cactus Pear Agro-Businesses

Although cactus pear is well-known to many South Africans, misconceptions about its potential persist. Efforts are therefore under-way to revitalise interest in the plant's production, promoting a renewed awareness of its versatility and multiple applications (Brown, 2020).

Currently fresh fruit is commercially produced and sold mainly in the Highveld area in South Africa. A main disadvantage is that the fruit's production and harvesting coincides with the production (mid-season) of many popular summer-produced fruits. This leads to many fruit being used as animal feed. This situation can be mitigated by production in colder areas in South Africa to produce fruit later in the summer season and at a higher price (Boraing, 2020; personal communication).

Most cactus pear producers are using the cladodes for animal feed. New interest to use cladodes for biogas is however emerging. There is currently approximately 300 ha under high-density plantations of cladodes for this application, with a predicted 1000 ha high-density plantations envisioned for the next year. Furthermore, cactus pear plants are used to rehabilitate mine soils, which in turn, can be applied in the biogas industry (Barren Energy, 2020; personal communication).

Increased interest in the food uses of cladodes are also noticed. This interest has led to the development and publication of a recipe book by the UFS in collaboration with the ARC and would soon be published on the UFS' website ([www.ufs.ac.za](http://www.ufs.ac.za)). Research collaboration between the UFS and the ARC has also led to the development of a cellphone application (App) called CactiGrow providing information on South African cactus pear cultivars and its cultivation.

Various restaurants, boutique hotels, guesthouses and road stalls are producing and selling unique cactus pear processed products. Some of these are internationally renowned, e.g. Babylonstoren in Stellenbosch, Western Cape. There is also a new

tendency for some of the farmers in some wine-producing areas to replace their vineyards with cactus plantations (Fouché, 2020; personal communication).

Another recently established industry is the seed oils (Cactus Goods Co.; personal communication). These are used in mostly skin-care products (Barnes, 2020; Personal communication).

## 6 Conclusion

In South Africa the outdated perception of cactus pears as thorny, alien invaders, is rapidly disappearing. Instead, farmers now recognize that cactus pear can play a vital role as a high yielding, water-efficient, multi-use crop.

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# Chapter 3

## Ethnobotany, Medicinal Utilization and Systematics of *Opuntia* Species from Deserts of Pakistan



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**Abstract** The genus *Opuntia* belonging to the family Cactaceae includes taxa, which are not sufficiently known from an ethnobotanical and systematics point of view in Pakistan. The present study is focused on taxonomic and comprehensive information on the floristic diversity and ethnomedicinal properties of cactus *Opuntia* plants. Detailed surveys of habitats containing naturalized populations of *Opuntia* spp. were made to select study sites. Although introduced at different times, they have adapted to the semi-arid environment and established in the wild for different uses. Based on field surveys and a literature review, we found an impressive population of an invasive *Opuntia* species. These semi-arid resistant plants are grown worldwide for its multifunctional proprieties. Research activities and comprehensive documentation on a different aspect of *Opuntia* species, e.g., *Opuntia dillenii*, *Opuntia ficus-indica*, and *Opuntia monacantha* from Pakistan, were provided about their morphology, geographic distribution, phenology, habitat, herbarium, and living materials. In addition, field collections (photographic records)

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were made. This study is also enriched by our observations on the palyno-anatomical features of three cactus *Opuntia* species. The ethnomedicinal studies on *Opuntia* spp. were accomplished to know about the species' traditional knowledge record and varieties of its uses by the folk peoples. Due to their economic importance, *Opuntia* will remain an interminable source of products with functions for food and livestock and contribute to the population's food security in marginalized areas. This book chapter summarizes current knowledge of *Opuntia* cacti's taxonomic studies, emphasizing its use as food and medicine.

**Keywords** *Opuntia* · Systematics · Ethnomedicinal properties · Palyno-anatomy

## 1 Introduction

### 1.1 Family Cactaceae

Cactaceae is angiosperms dicotyledonous plants family of approximately 2260 total accepted taxa; 1306 species; 301 accepted taxonomic subspecies; 582 provisionally recognized hybrids species, and 71 taxonomic subspecies (Labra et al., 2003). The family Cactaceae is a part of the order Caryophyllales classified into four subfamilies viz. Opuntioideae, Cactioideae, Pereskioideae, and Maihuenioideae recognized based on the morphology of vegetative and floral parts (Anderson, 2001; Labra et al., 2003). Cactus are naturally found in New World regions with dry and semi-desert conditions (Nyffeler, 2002). The Cactaceae members have a cylindrical, globular, or flat stem (cladode) (Salgado and Mauseth, 2002). Cactus plants riveted a unique consideration because of their particular properties of the bristly succulent body and crassulacean acid metabolism (CAM) for water storage and photosynthesis, which allow them to live in extreme environmental conditions and bear water stress (Ortega-Baes et al., 2010). The trade-in cactus plants worldwide are of vital importance for their propagation. The deliberate introduction of seed companies, nurseries, and botanical gardens in recent years has grown into industry significantly (Novoa et al., 2016). In the old world, cacti are cultivated distinctly as an ornamental plant (Garralla and Cuadrado, 2007). Cactus familiarized in many areas of the world for human utilization, animal fodder, medicinal aspects, and ornamental purposes that are vital economically (Novoa et al., 2015).

### 1.2 Subfamily Opuntioideae

Opuntioideae is a traditionally recognized subfamily in Cactaceae, representing approximately 220–350 species with a tree-like, shrub-like, or climbing habit, distributed in Central and South America, North America, the Caribbean, and Canada. Opuntioideae have been conventionally regarded as a monophyletic taxonomic

group (Barthlott et al., 2001; Ritz et al., 2012). A variety of synapomorphic derived traits distinguishes Opuntioideae: (a) presence of small, deciduous barbed spiny glochidia (Las Peñas et al., 2019), (b) seed enclosed in woody funicular tissue (Stuppy, 2002), (c) high concentration of calcium oxalate monohydrate druses and monoclinic cluster crystals found in the outer hypodermis of stem (Hartl et al., 2007), and (d) characteristic types of exine ornamentation in polyporate pollen grains (Long, 2012).

### 1.3 Genus *Opuntia* Mill.

*Opuntia* Mill., the most speciose genus in the Opuntioideae subfamily, comprises 181 species with ten natural hybrids (Barthlott et al., 2001). The first worldwide classification of *Opuntia* was proposed by Britton and Rose (1919), which is the largest genus in the Cactaceae family, including several species of dicot angiosperm (Galvez et al., 2009; Melgar et al., 2017a). The genus *Opuntia* has been reported to have nearly 300 different varieties between domesticated and wild species (Melgar et al., 2017b). The *Opuntia* genus includes many problematic taxa that have been recorded as naturalized or invasive in many countries of Asia, the Middle East, Europe, Australia, and Africa (Shackleton et al., 2017). Members of the genus *Opuntia* are; (1) bushy or shrubby plants with a jointed stem consisting of fleshy, elliptical, and flattened pads, (b) the presence of pleasing, delicious edible fruits, (c) the areoles with small, easily detachable prickly spines, (d) rudimentary leaves on newly developed phylloclades, and (e) the seeds with light color integument (Feugang et al., 2006; Arba et al., 2017). Cactus *Opuntia* is tropical plant species capable of growing under arid and semi-arid conditions, with CAM metabolism readily adaptable to their geography. CAM helps the plant to withstand enables extreme weather, low temperature, and drought because of the higher efficiency of their water usage (Sudzuki et al., 1993; Nobel and Castañeda, 1998; Sepúlveda et al., 2007). *Opuntia* flowers are auxiliary, wide, bisexual, and have spirally arranged tepals come in different colors of development begin from white through yellow to red, orange, pink, peach, and cream (Heuzé and Tran, 2017). *Opuntia* species distributed worldwide have been widely used as a medicine, forage, foodstuffs, and ornamental plants (Silva-Hughes et al., 2015). *Opuntia* plants are the place of origin for cosmetics and pharmaceutical products (Felker and Inglese, 2003). Cactus *Opuntia* plants contain different phytochemicals such as amino acids, vitamins C and E, carotenoids, antioxidant phenolic components (betalains and flavonoids), phenolic acids, sterols, esters, coumarins, terpenoids, and alkaloids that carry out several medicinal benefits such as hypoglycemic activity, antioxidant activity, anti-inflammatory activity, immune-stimulatory effect, free radical-scavenging activity, anti-tumor, blood lipid-lowering effect, anti-diabetic and wound healing activity (Alarcon-Aguilar et al., 2003; Ahmed et al., 2005; Cho et al., 2006; Trombetta et al., 2006; Panico et al., 2007; Huang et al., 2008; Schepetkin et al., 2008). The prickly pear fruit extract is commonly used in folk medication for burned wound, oedema, and considered to have a better effect than stem extract (Shetty et al., 2012).

Additionally, fruit and mature stem cladodes are edible foods for humans, and livestock was contributed to population food security in marginalized areas (Maki-Díaz et al., 2015). Because of their economic importance, many *Opuntia* species are marketed in several countries including Mexico, United States, Chile, Argentina, Italy, Spain, and South Africa as either exotic fruit, vegetables, or forage (Galvez et al., 2009).

#### 1.4 Ethnomedicinal Properties

The *Opuntia* spp. vegetative parts are used traditionally as a valuable health promotive nutrient and rarely used in modern medicine and diet. It is also essential in the pharmaceutical industries (Shetty et al., 2012). Studies described that *Opuntia dillenii* cladodes as well as fruits having several health-promoting activities. They are considered edible, safe, and non-toxic, generally used as a fresh or processed vegetable for human consumption. The stem cladodes are used in folk medication to treat asthma, diabetes, gastric ulcers, ophthalmia, inflammation, hepatitis, and decreasing cholesterol levels and intestinal spasm (Chang et al., 2008; Siddiqui et al., 2016a; Kalegowda et al., 2017). *Opuntia dillenii* has been widely used to prepare as a vegetable and as a preservative in food. In many countries, *O. dillenii* has also been used traditionally as an herbal remedy for detumescent drainage promoter, promoting blood circulation through anti-inflammatory and antioxidative activities (Moussa-Ayoub et al., 2017). The cladode segments of *O. dillenii* exhibited wide-ranging pharmacological actions, such as immunostimulatory, hypoglycemic, anti-microbial, analgesic, hypotensive, hypoglycemic, promote wound healing and anti-asthmatic effects (Li et al., 2016; Siddiqui et al., 2016a; Siddiqui et al., 2016b). The polysaccharides fraction from cladodes of *O. dillenii* showed hypolipidemic, neuroprotective, and anti-diabetic effects (Li et al., 2014; Gao et al., 2015). Antioxidant activities of *O. dillenii* significantly reduce the oxidative stress in patients of chronic pathologies (Shah et al., 2016).

*Opuntia ficus-indica* produced sweet, nutritious, rich edible fruits and its succulent stem cladodes are used as fresh green vegetables. The fruit, as well as cactus cladodes, are used for the preparations of better quality products such as jam, squash, wine, pickle, body lotions, shampoo, creams, and its seeds could be used as agents of flavoring (Pareek et al., 2003; Kaur et al., 2012). The herbal extracts of its stem cladode exhibited anti-diabetic, anti-inflammatory, hypocholesterolemic, hypoglycemic, and hypolipidemic effects. Moreover, the fruit contains betalains and phenolic compounds in abundance that are well-known for their antioxidant properties (Otálora et al., 2015). *Opuntia ficus indica* (cactus pear) used as a folk medicinal plant in different regions of the world for treating the burns, wounds, edema, and indigestion. The cladodes extract of *O. ficus indica* have shown anti-ulcer and wound healing activities (Trombetta et al., 2006). Additionally, the prickly pear's stem segments have traditionally been used to treat diabetes (Tilahun and Welegerima, 2018). The flower infusion of *O. ficus-indica* is used as a traditional

remedy, showing a depurative effect, which is mainly used for the renal excretory pathway for its diuretic and relaxant action (Galati et al., 2002).

*Opuntia monacantha* showed various pharmacological properties including emollient, hypoglycemic, and hypocholesterolemic effects through their anti-inflammatory and antioxidant actions (Yang et al., 2008). It has also been extensively used as home medication; has effective better analgesic actions, blood circulation, and detoxification (Kwon et al., 2017). The isolated polysaccharides from *O. monacantha* stem cladodes showed anti-diabetic and anti-glycated activities. *Opuntia monacantha* also exerted inhibition of stomach ulcer and neuroprotective effects (Yang et al., 2008; Bari et al., 2012). The *O. monacantha* stem segments are recommended for their beneficial therapeutic effects and has been traditionally used as folk medicine; for the treatment of anemia, bronchial asthma, burns, diabetes, indigestion, inflammations, spleen enlargement, ulcers and tumors in the urinary tract (Zhao et al., 2007; Kifayatullah and Waheed, 2014). The flowers have been used for the ophthalmic and respiratory infections, while fruit used for treating gonorrhoea (Saleem et al., 2015).

### 1.5 Palyno-Anatomical Studies

In Cactaceae, most researchers have focused diversification of epidermal cells in one genera with few species or different subfamilies or tribes with several genera (Eggl, 1984; Loza-Cornejo and Terrazas, 2003; Soffiatti and Angyalossy, 2007; Calvente et al., 2008; Faigón et al., 2011). The cactus surface outline provided an essential barrier between the plant and a frequently hostile environment. On this subject, unexpectedly, a little taxonomic work is done, and from the viewpoint of taxonomic value, it is subject of interest (Gasson, 1981). The outline of classification at different levels of hierarchy are the major source of data in systematics for macro and micro-morphological diversity of epidermal cells (Nishida and van der Werff, 2007; Lu et al., 2008; Cervantes et al., 2009; Dunthorn, 2009; Araújo et al., 2010).

According to earlier studies, *Opuntia* species has been investigated for wood anatomy, wideband tracheid's anatomy, and epidermal anatomical characters (Mauseth, 2004, 2006; Faigón et al., 2011), but no data was recorded for stem anatomical features of *Opuntia* species in detail from Pakistan. *Opuntia*'s stomatal complex belongs to the paracytic type, a common feature in the Opuntioideae group. The important taxonomical feature for subunit Opuntioideae is the stomata, classified as "*opuntioid*" the characterized by the number of undetermined subsidiary cells around the guard cells (Eggl, 1984).

Pollen morphological traits may be beneficial in the accurate identification of complex taxa of the *Opuntia* genus due to resemblance in their morphological characters. Therefore, the *Opuntia* species has been examined palyno-morphologically (Leuenberger, 1976; Garralla and Cuadrado, 2007; ElBehi et al., 2013; Majure and

Puente, 2014; Martínez-González et al., 2019). According to these investigations, pollen within this genus are highly variable in shape, apertural features, sculpture pattern, and size. Generally, grains are spherical, apolar, radio-symmetric, circular, polyhedral hexagonal, circular polygonal, polyporate surface with tectate or semi tectate, spinulose perforate and reticulate peculiarities of exine tectum.

The present research work aims to collect the fresh specimens of *Opuntia* species from different areas of Pakistan for their correct identification and taxonomic description. Furthermore, an advanced study of *Opuntia* diversity was investigated. Morpho-anatomical and pollen micro-morphological investigations provided valuable information for the delimitation of species, their accurate identification, and also to fix the taxonomic problems. The present work may be effective for morphological, stem anatomical, and palynological findings of *Opuntia*. The study may also provide ethnobotanical documentation about the medicinal uses of *Opuntia* species from the research areas.

## 2 Methodology

### 2.1 Plant Sampling

For research work, *Opuntia*'s plant specimens were collected in wild conditions from different Pakistan's localities, including Islamabad Capital territory, District Attock, and Mianwali. For the systematic studies from different localities of the research area, plant samples, along with their stem and flower, were collected, and local people were interviewed for the collection of ethnomedicinal data documentation. Each plant sample was numbered, and their macro photography during collection was done on spot. The geographical information and coordinates were recorded using GPS during the field survey. After the specimen, drying in the sun was preserved in the herbarium and the botanical name, collection date, and habitat record were recorded. In Table 3.1, *Opuntia* plant species arranged alphabetically along with locality, coordinates, altitude, and voucher specimen number.

**Table 3.1** Plant sampling and vouchering

Sr. No.	Taxa	Voucher specimen no.	Locality	Coordinates
01	<i>Opuntia dillenii</i> (Ker Gawl.) Haw.	ISL-SMC-7	Kundian (Mianwali)	32° 27' 28N 71° 29' 44E
02	<i>Opuntia ficus-indica</i> (L.) Mill.	ISL-SMC-1	Chhab (Attock), QAU Islamabad	33° 27' 15N 71° 54' 5E
03	<i>Opuntia monacantha</i> (Willd.) Haw	ISL-SMC-8	Teri Khel Mianwali	32° 41' 17N 71° 32' 33E

## 2.2 *Preservation and Identification*

The collected specimens of plants according to standard herbarium techniques were preserved in their original shape. The plants were dried by leaving the stem sections in the shade or slicing the specimens to remove inner parenchyma then mounted on herbarium sheets (De Groot, 2011). Field identification of *Opuntia* spp. under different ecological conditions at multiple locations was carried out to study their gross morphology. For their correct identification, in addition to extensive field investigations, we consulted herbarium specimens' collections, extensive literature surveys, original documentation, and description from different flora of the world ([www.efloras.org](http://www.efloras.org)). The correct plant names of *Opuntia* species were confirmed from The Plant List ([www.theplantlist.org](http://www.theplantlist.org)) and IPNI ([www.ipni.org](http://www.ipni.org)).

## 2.3 *Poisoning and Mounting*

Plant samples' poisoning was carried out after drying using a solution of 16 g of mercuric chloride mixed in 1 L ethanol. Approximately 1–2 min, plant samples were dipped in the solution, then dryness of specimens was carried out and then put specimens in the newspaper. After that, on herbarium sheets of standard size (42 cm × 28 cm), *Opuntia* specimens were mounted. The herbarium labels were also mounted on the lower right-side of sheets having information about species name and their family including collection date, province, district, locality, and the name of the person who identifies the plant species.

## 2.4 *Morphological Studies*

*Opuntia* species are very much similar to each other morphologically, and no complete information is present on the morphology of these plants. Firstly, plant specimens were carefully handled using forceps and then placed on a wooden piece to observe morphometric parameters. Qualitative morphological characters (surface, shape, areoles, spines, glochids, and fruit) were observed with the naked eye, magnifiers (10×, 20×, and 40×) and also studied under dissection binocular microscope (Carl Zeiss Model W 10×/20L). Stem quantitative characters, i.e., stem dimensions, spine length, and fruit size, were noted with measuring scales. The stem and spine readings were taken very carefully by putting hand gloves. The measurement of whole specimens was mostly taken, and their mean value was calculated.

## 2.5 Anatomical Investigation

For the epidermal section's stem anatomy, *Opuntia* stem 3–4 small bits were removed with a sharp razor blade, put in a test tube containing 65% lactic acid, and 35% nitric acid depend upon the thickness of the stem segments of different species (Table 3.2). The test tube was heated on flame for 2–3 min, and drops of water were added. The transparent sections emptied into the petri dish were washed with water twice to remove chemicals and separated with camel hairbrushes. Afterward, transparent epidermis sections were placed on a glass slide treated with lactic acid drops; the coverslip was put on it. The deposition of nail polish to make the slides permanent was done along the slide and coverslip margins. The anatomical characters (epidermal cell, guard cells, stomata, subsidiary cells, and trichomes) under light microscope prepared slides were examined. The stem section's microphotographs were taken using an LCD microscope (Model: XSP-45) at 10× magnification.

**Table 3.2** Anatomical features of cactus *Opuntia* species

Anatomical Character	<i>Opuntia dillenii</i> (Ker Gawl.) Haw.	<i>Opuntia ficus-indica</i> (L.) Mill	<i>Opuntia monacantha</i> (Willd.) Haw.
Epidermal cell shape	Irregular	Polygonal	Zigzag
Anticlinal wall	Straight	Sinuuous	Sinuate
Stomata	Paracytic	Paracytic	Paracytic
Guard cell shape	Kidney shape	Kidney shape	Kidney shape
Epidermal cell L (µm) Max – Min = M ± SE	21.75–53.50 = 37.40 ± 6.25	26.5–42.75 = 36.55 ± 2.82	46.75–58.50 = 53.25 ± 2.05
Epidermal cell W (µm) Max – Min = M ± SE	18.25–39 = 27.85 ± 4.17	24.25–37 = 30.95 ± 2.15	21.25–48.75 = 35.10 ± 5.19
Stomata L (µm) Max – Min = M ± SE	33.25–40.50 = 37.35 ± 1.20	46.5–55.25 = 50.95 ± 1.39	48.50–56 = 52.95 ± 1.39
Stomata W (µm) Max – Min = M ± SE	13.50–20.50 = 17.60 ± 1.20	20.25–23 = 21.60 ± 0.54	8.50–11.25 = 9.60 ± 0.55
Stomatal pore L (µm) Max – Min = M ± SE	23–35.75 = 28.60 ± 2.14	11.75–16.5 = 13.50 ± 0.80	21.50–28.50 = 24.30 ± 1.23
Stomatal pore W (µm) Max – Min = M ± SE	12.75–16 = 14.15 ± 0.63	3.25–4.50 = 3.75 ± 0.20	13.50–17.75 = 15.35 ± 0.78
Stomatal index (%)	3.31	8.07	3.23

Key words: *L* length, *W* width, *M* mean, *SE* standard error, *Max* maximum, *Min* minimum, *µm* micrometer

## 2.6 *Morpho-Palynological Study*

### 2.6.1 **Light Microscopy (LM)**

The method of Erdtman (1966) was followed, anthers removed from filaments with the help of dissecting needles placed on a glass slide was treated with 1–2 drops of acetic acid. After that, crushed to release pollen on the slide were stained with glycerin jelly prepared (Zafar et al., 2006). The coverslip was put on it and make it permanent with transparent nail varnish. Observing morphological parameters for ten randomly selected pollen grains (size, type, pollen diameters, apertures length and width, and exine thickness), under light microscope prepared slides were studied. Their micrographs were taken at 20× and 40× magnification with a photomicrograph system equipped with Nikon FX-35 Camera.

### 2.6.2 **Scanning Electron Microscopy (SEM)**

The Erdtman (1952) technique for scanning electron microscopic investigation was used to prepare pollen slides. Firstly, anthers separated from flowers and crushed to release the pollen, treated with 3–4 drops of 45% acetic acid, and then mounted directly on prepared stubs. Afterward, acetolyzed pollen samples sputtering was done with gold-palladium coating and then observed for micromorphological pollen characters under scanning microscope (Model JEOL JSM 25910) in Central Resource Laboratory (CRL), the University of Peshawar, Pakistan (Mir et al., 2019).

## 2.7 *Numerical Analysis*

### 2.7.1 **Stomatal Index**

The stomatal index calculation was done using the formula (Ullah et al., 2018b; Zafar et al., 2019)

$$\mathbf{S.I = S / E + S \times 100} \quad (3.1)$$

where, S.I =Stomatal index; E =epidermal cell number per unit area; S =number of stomata per unit area.

### 2.7.2 **Pollen Fertility and Sterility**

The pollen fertility and sterility were estimated using the formula of Ullah et al. (2018a) (Table 3.3).



**Table 3.3** Pollen morphological attributes of *Opuntia* species

Palynological attribute	<i>Opuntia dillenii</i> (Ker Gawl.) Haw.	<i>Opuntia ficus-indica</i> (L.) Mill	<i>Opuntia monacantha</i> (Willd.) Haw.
Equatorial view shape	Prolate spheroidal	Prolate spheroidal	Prolate spheroidal
Pollen type	Pantoporate	Pantoporate	Pantoporate
Aperture orientation	Slightly sunken	Sunken	Sunken
Exine ornamentation	Perforate reticulate	Reticulate	Reticulate semitectate
Polar diameter ( $\mu\text{m}$ ) Max–Min = M $\pm$ SE	110–132.50 = 121.4 $\pm$ 2.31	101–130.75 = 116.95 $\pm$ 2.83	97.50–127 = 112.08 $\pm$ 3.35
Equatorial diameter ( $\mu\text{m}$ ) Max–Min = M $\pm$ SE	84.25–132.5 = 108.3 $\pm$ 4.6	103.5–120.25 = 112.2 $\pm$ 1.7	90–115 = 101.65 $\pm$ 2.71
P/E ratio	1.12	1.04	1.10
Aperture length ( $\mu\text{m}$ ) Max–Min = M $\pm$ SE	15–27.25 = 20.37 $\pm$ 1.30	13.25–22 = 18.62 $\pm$ 0.98	17.5–27.50 = 22.10 $\pm$ 0.95
Aperture width ( $\mu\text{m}$ ) Max–Min = M $\pm$ SE	12.5–23.50 = 18.50 $\pm$ 1.06	9.50–12.25 = 10.95 $\pm$ 0.26	7.50–20 = 13.22 $\pm$ 1.32
Exine thickness ( $\mu\text{m}$ ) Max–Min = M $\pm$ SE	5.50–13.50 = 9.90 $\pm$ 0.79	5.50–7 = 6.17 $\pm$ 0.19	5–12.50 = 8.57 $\pm$ 0.76
Pollen fertility (%)	72.10	57.89	77.22
Pollen sterility (%)	27.89	42.10	22.77

Key words: *P* polar diameter, *E* equatorial diameter, *M* mean, *SE* standard error, *Max* maximum, *Min* minimum,  $\mu\text{m}$  micrometer

$$\text{Fertility} = \mathbf{F / F + S \times 100} \quad (3.2)$$

$$\text{Sterility} = \mathbf{S / S + F \times 100} \quad (3.3)$$

F = number of fertile pollen on the same ocular, and S = number of sterile grains on the same ocular.

## 2.8 Ethnobotanical Data Collection

The ethnobotanical study was carried out at different sites of research areas during field trips to gather the field notes. A total of 40 informants were interviewed, including males and females, and their ages ranged between 30 and 76 years. Commonly most of the informants were not highly educated. Peoples, including folk peoples and local vendors, were interviewed to ask about the cactus *Opuntia* species concerning their ethnomedicinal usage.

### 2.8.1 Quantitative Analysis

The ethnobotanical documentation collected through the semi-structured interview form informants was analyzed quantitatively using various relative indices (a) Use value, (b) Relative frequency of citation, and (c) Informant consensus factor.

#### (a) Use Value (UV)

The procedure of Yasin et al. (2019) was apply for the calculation of use-value

$$UV = \sum U / n \quad (3.4)$$

where U is the total number of use report cited for a given plant species, and n mention about the total number of informants investigate for given *Opuntia* species. If the use-value is close to zero, the UV is low, demonstrating few use reports for given plant species.

#### (b) Relative Frequency of Citation (RFC)

RFC was used to analyze the indigenous status of the medicinal plant from collected ethnomedicinal data quantitatively, and the index demonstrates the importance of local species

$$RFC = FC / N (0 < RFC < 1) \quad (3.5)$$

It is represented by the frequency of citation (FC); is the number of informants mentioning the use of plant species, and N indicates the total number of informants taking part in surveys without use categories (Sargin et al., 2015).

#### (c) Informant Consensus Factor (ICF)

ICF index was determined using the given formula of Sargin et al. (2013).

$$ICF = \frac{Nur - Nt}{Nur - 1} \quad (3.6)$$

Nur mentions the number of use reports for a specific disease category, and Nt indicates the number of species used for disease category. The medicinal plants have high ICF values treating a certain ailment very effectively (Teklehaymanot and Giday, 2007).

### 3 Results

#### 3.1 Systematic Findings of *Opuntia* Species

The present research reported three *Opuntia* plants species investigating their morphological, stem anatomical, and palyno-morphological features using different microscopic techniques. *Opuntia dillenii*, *Opuntia ficus-indica*, and *Opuntia monacantha* were collected from a different Pakistan region. The investigated *Opuntia* species were arranged in alphabetical order with species description, including species name, synonym, cosmopolitan distribution, habitat, vernacular name, common names, and flowering period.

The taxonomic appraisal of *Opuntia* species using morphological characters is beneficial in the correct identification, and species delimitation of this genus is very important in evolution. This research also elaborates on the macro morphological characters examined through the naked eye using a magnifying lens and under dissecting binocular microscope. The detailed morphological description of *Opuntia* species including length and width of a plant, stem shape, stem segment (phylloclade or cladode) size, spines size, areoles features, glochids, flower description included calyx, sepals, petals, and style and fruit explanation was noted.

The current study examines the considerable variations in stem anatomical characters both qualitatively and quantitatively under the light microscope. The stem epidermal sectioning shows apparent variations in epidermal cell size, number, shape, wall pattern, stomata type and size, stomatal pore, guard cells, subsidiary cells, and trichomes features. Stem anatomical features are basically similar in the present findings. However, they show that many diverse characters are of special attention for plant taxonomists to correctly identify and provide a baseline for further study to determine the systematic position of *Opuntia* species.

This study also focused on exploring the micromorphological pollen features of *Opuntia* species using light and scanning electron microscopy. Detailed palynological attributes such as pollen diameter, size, type, shape, aperture size and orientation, exine sculpturing of three plant taxa were investigated. The detailed pollen morphological analysis has many valuable taxonomic characters to understand their systematics better and play a significant role in the accurate identification of *Opuntia* species.

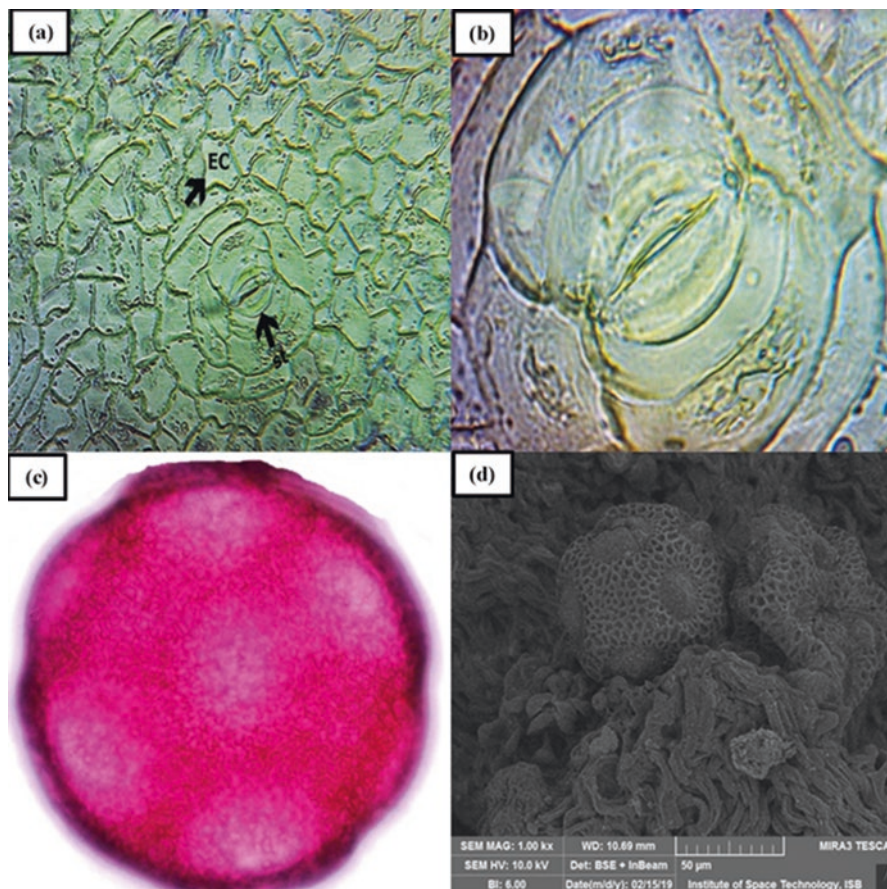
##### 3.1.1 *Opuntia dillenii* (Ker-Gawl) Haw (Figs. 3.1 and 3.2)

<b>Synonym</b>	:	<i>Cactus chinensis</i> Roxb.
<b>Common Name</b>	:	Erect prickly pear
<b>Vernacular Name</b>	:	Thor

<b>Habitat</b>	: Terrestrial, along the roadside
<b>Worldwide Distribution</b>	: Ecuador, Mexico, South Carolina, Texas, and Florida
<b>Distribution in Pakistan</b>	: Rawalpindi, Attock, Mianwali, Buner, Karak, and Kotli
<b>Phenology</b>	: May to July



**Fig. 3.1** *Opuntia dillenii* (a) stem cladodes, (b) flower, (c) fruit, and (d) preserved herbarium specimen



**Fig. 3.2** Microscopic photographs of *Opuntia dillenii* showing (a) stem epidermal surface, (b) stomata, (c) light microscopic pollen, and (d) SEM pollen view

<b>Morphological Description</b>	<p>: A xerophytic evergreen shrub is found in drying conditions, perennial, often in the form of dense clumps, usually growing 45–90 cm tall. Stem segments (phylloclade or cladodes) elliptical, oblong, and flattened in shape 10–22 cm long and 6–17 cm wide. Spines: varies greatly, yellow, usually 3–7, curved and straight, 13–24 mm long and 1.5–2.2 mm wide, areoles; large, prickly, yellow, 11–17 mm long with glochidia (short barbed bristles) in the base. Flowers: yellowish orange to lemon yellowish in color, cyclic, 5–7 cm long and 5.5–8.5 cm across., perianth rotate and lobed, androecium versatile, numerous petals and stamens, lobed stigma and an inferior ovary. Fruits: fleshy, obovoid, edible, purple, pyriform, apically depressed, 4 × 9 cm × 2–3.5 cm in diameter; with tufts of tiny barbed spines glochids</p>
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<b>Stem Anatomy</b>	: The shape of epidermal cells are irregular, straight anticlinal wall, the length of epidermal cells is $37.40 \pm 6.25 \mu\text{m}$ , and the width of cells is $27.85 \pm 4.17 \mu\text{m}$ . The average epidermal cell number is 124 per unit area. The mean value of no. of stomata is 4 per unit area. Stomata length and width is $37.35 \pm 1.20 \mu\text{m}$ and $17.60 \pm 1.20 \mu\text{m}$ , respectively. Guard cells noted length is $50.95 \pm 1.22 \mu\text{m}$ and breadth is $11.80 \pm 0.52 \mu\text{m}$ . Subsidiary cells length measurement is $52.90 \pm 1.19 \mu\text{m}$ , and the width is $14.15 \pm 0.63 \mu\text{m}$ . The length of the stomatal pore is $28.60 \pm 2.14 \mu\text{m}$ , and the width is $11.80 \pm 0.52 \mu\text{m}$ . Stomata are of a paracytic type. The kidney shape of the guard cell is observed. The Stomatal index has a value of 3.31. Trichomes absent
<b>Pollen Morphology</b>	: Pollen monad, large, pantoporate, and psilate. Pollen shape circular in polar view, in equatorial view; prolate spheroidal. The polar and equatorial diameter was found to be $121.4 \pm 2.31 \mu\text{m}$ and $108.3 \pm 4.63 \mu\text{m}$ , respectively. Colpi length was measured $20.37 \pm 1.30 \mu\text{m}$ , and the calculated colpi width was $18.50 \pm 1.06 \mu\text{m}$ . The thickness of the exine was calculated $9.90 \pm 0.79 \mu\text{m}$ . The ratio of polar to equatorial diameter (P/E) was found to be 1.12. No of fertile pollen measured was 137, and sterile pollen was 53. Pollen fertility estimated was 72.10%

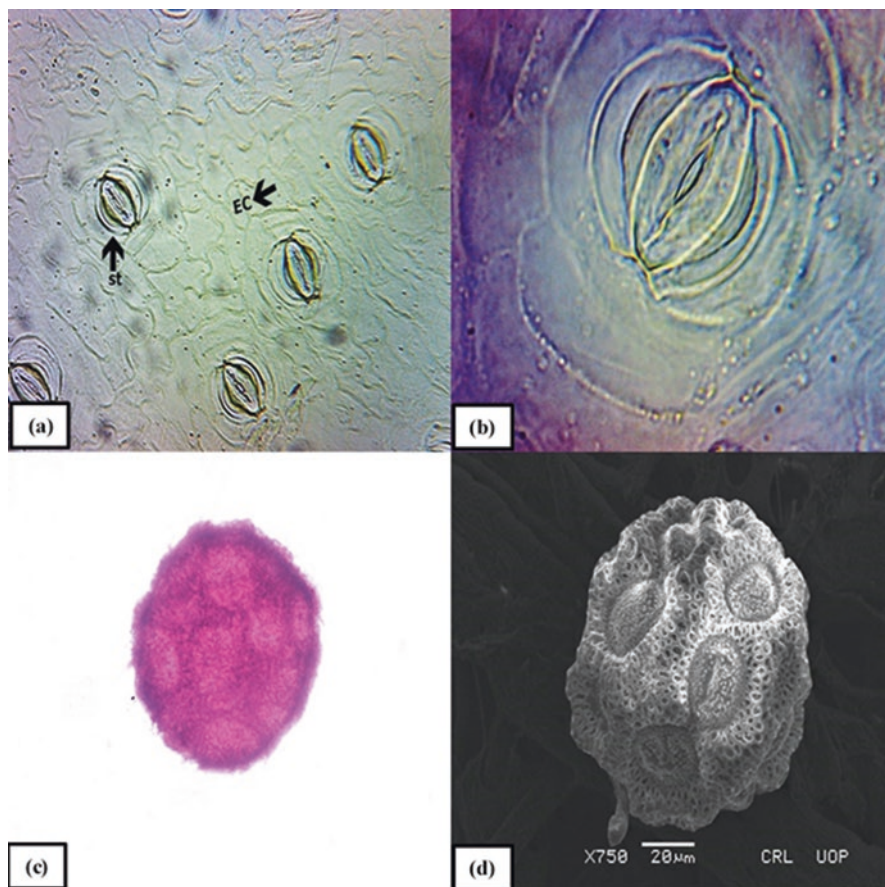
### 3.1.2 *Opuntia ficus-indica* (L.) Mill (Figs. 3.3 and 3.4)

<b>Synonym</b>	: <i>Cactus ficus-indica</i> L.
<b>Common Name</b>	: Barbary fig
<b>Vernacular Name</b>	: Danda Thohar
<b>Habitat</b>	: Pastures and along the roadside
<b>Worldwide Distribution</b>	: Algeria, Brazil, Chile, Eritrea, India, Uganda, Italy, Kenya, Portugal, Spain, Tunisia, Morocco, Ethiopia, and Mexico
<b>Distribution in Pakistan</b>	: Mianwali, Islamabad, Talagang, Karak, Mirpurkhas, Attock
<b>Phenology</b>	: End of April to June
<b>Morphological Description</b>	: <i>O. ficus-indica</i> is a slow-growing, evergreen, large, bushy, perennial segmented cactus which can attain a height of 3–6 m high and up to 1 m in diameter. <b>Stem</b> cladodes are succulent, flattened broadly obovate, oblong to spatulate, green to blue-green, 12–26 cm long, and 8–20 cm wide. Spines: inconspicuous, present or absent, straight, flattened, 7–16 mm long, 2–4 mm broad, areoles; small, elliptical, white or pale brown, numerous yellow glochids, protruding 1–2 mm. Flowers: large 5.5–7 cm long and 4–7 cm broad, yellow to orange-yellow, cup-shaped, formed on the edges of cladodes, anthers and filaments yellow, stigma lobed and style greenish. The fruit is reddish, succulent, oblong, 7–10 cm long, 3–8 cm broad, fleshy, juicy, edible with glochids present on the surface



**Fig. 3.3** *Opuntia ficus-indica* (a) stem cladodes, (b) flower, (c) fruit, and (d) preserved herbarium specimen

<p><b>Stem Anatomy</b></p>	<p>: The shape of epidermal cells: wavy and polygonal, sinuous anticlinal wall, epidermal cells length, and breadth is <math>36.55 \pm 2.82 \mu\text{m}</math> and <math>30.95 \pm 2.15 \mu\text{m}</math>, respectively. The average number per unit area of epidermal cells is 105. The mean value of the stomata number per unit area is 9. Stomata paracytic, the stomatal length is <math>50.95 \pm 1.39 \mu\text{m}</math> and width is <math>21.60 \pm 0.54 \mu\text{m}</math>. The length of the subsidiary cell is <math>55.70 \pm 1.31 \mu\text{m}</math>, and the width is <math>13.80 \pm 1.41 \mu\text{m}</math>. Guard cell length and width is <math>52.75 \pm 1.57 \mu\text{m}</math> and <math>10.80 \pm 0.21 \mu\text{m}</math>, respectively. The length of the stomatal pore is <math>13.50 \pm 0.80 \mu\text{m}</math>, and the width is <math>3.75 \pm 0.20 \mu\text{m}</math>. The stomatal index value is 8.07%. Non-glandular unicellular trichomes present. Examined trichome length is <math>92.62 \pm 5.59 \mu\text{m}</math>, and width is <math>7.32 \pm 1.77 \mu\text{m}</math></p>
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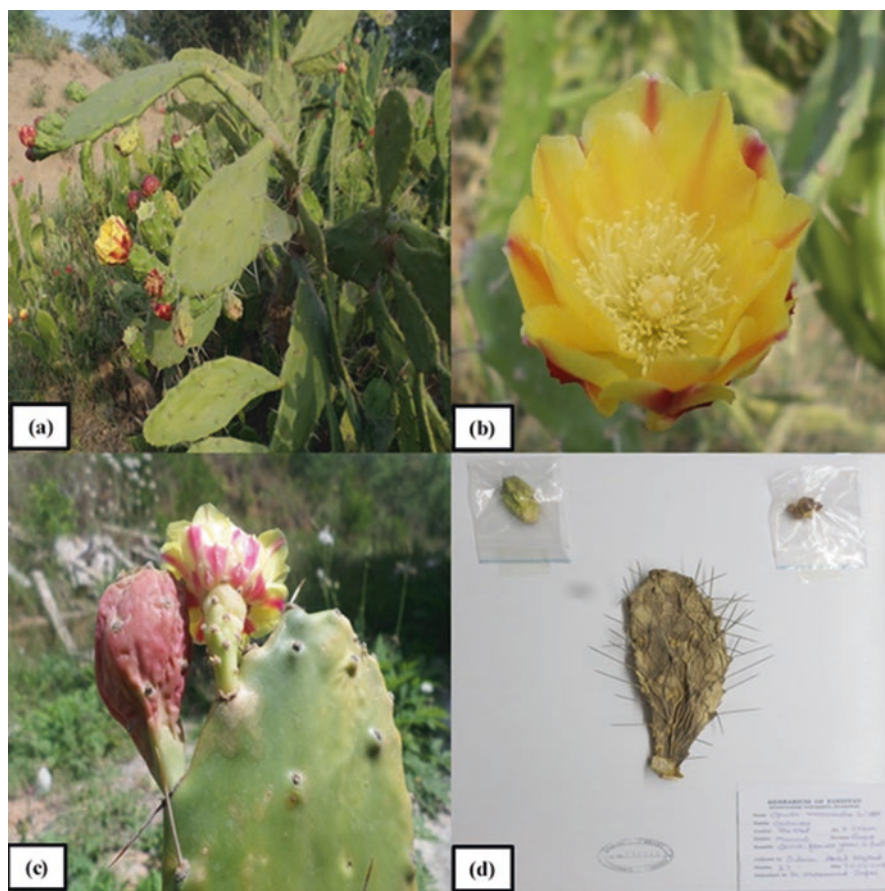
**Fig. 3.4** Microscopic photographs of *Opuntia ficus-indica* showing (a) stem epidermal surface, (b) stomata, (c) light microscopic pollen, and (d) SEM pollen view

<b>Pollen Morphology</b>	: Qualitatively the pollen grain is described as large, a polar monad, pantoporate, psilate-the pollen shape in polar view; circular and in equatorial view; prolate spheroidal. The polar diameter is $116.95 \pm 2.83 \mu\text{m}$ . The equatorial diameter is $112.27 \pm 1.75 \mu\text{m}$ . The length of colpi is $18.62 \pm 0.98 \mu\text{m}$ . The width of the colpi is $10.95 \pm 0.26 \mu\text{m}$ . Exine thickness $6.17 \pm 0.19 \mu\text{m}$ . P/E ratio of 1.12. No. of fertile pollen 55 and sterile pollen 40. The fertility of pollen is 57.89%
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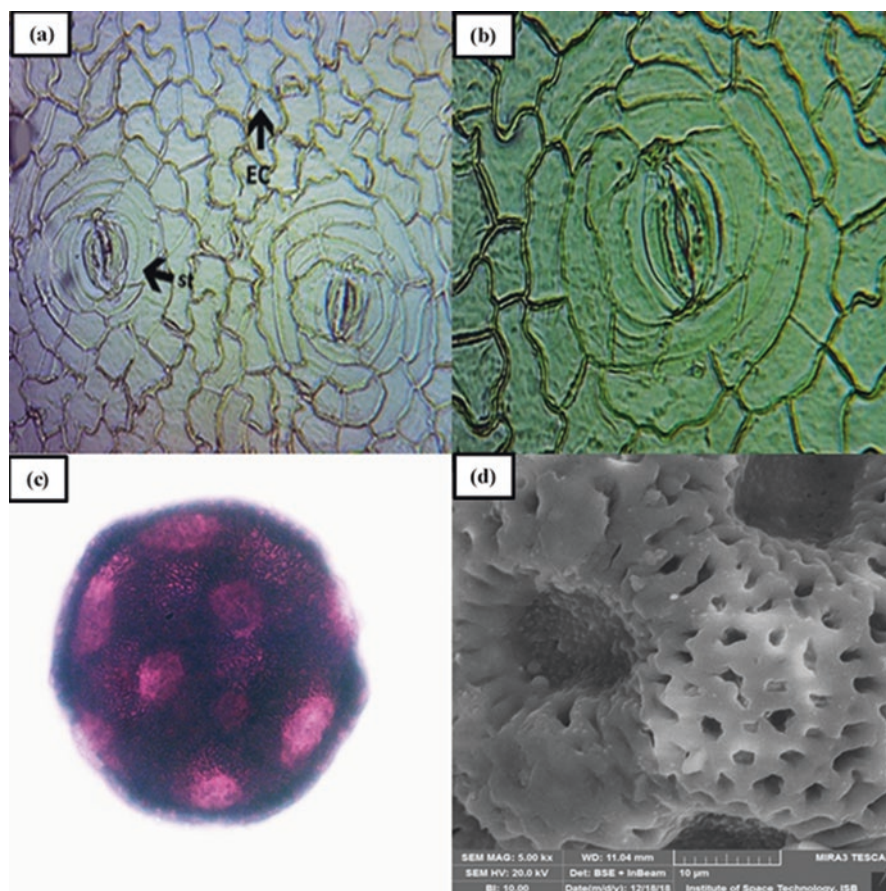


### 3.1.3 *Opuntia monacantha* (Willd.) Haw (Figs. 3.5 and 3.6)

<b>Synonym</b>	: <i>Cactus monacanthus</i> Willd.
<b>Common Name</b>	: Drooping prickly pear
<b>Vernacular Name</b>	: Nagphana
<b>Habitat</b>	: Waste sandy soil
<b>Worldwide Distribution</b>	: Waste sandy soil Uruguay, Argentina, Paraguay, Brazil, Australia, South Africa, Indonesia, Philippines, South-East Asia
<b>Distribution in Pakistan</b>	: Chakwal, Malakand, Lower Swat, Kohala, Domel and Faisalabad



**Fig. 3.5** *Opuntia monacantha* (a) stem cladodes, (b) flower, (c) fruit, and (d) preserved herbarium specimen



**Fig. 3.6** Microscopic photographs of *Opuntia monacantha* showing (a) stem epidermal surface, (b) stomata, (c) light microscopic pollen, and (d) SEM pollen view

<b>Phenology</b>	: May to August
<b>Morphological Description</b>	: Drooping prickly pear cactus, erect, succulent, tree-like plant, with upper branches drooping, up to 1–3 m tall. Stem: green, flattened, elongated, obovate shape, thin, fleshy, shiny, glabrous, and 13–37 cm in length and 6–15 cm in width. Spines: segments bear one larger spine (1.5–7 cm long), slender, greyish white in color with tips are darker, areoles; broadly separated, 8–23 mm long, and glochidia are absent. Flowers: showy, solitary, present along margins, yellow-reddish in color, 5–8.5 cm broad and 4.5–7 cm long, reddish outer segments of the perianth, with greenish-white stamen filaments. Fruit: conical or obovoid, green immature, purplish-red on maturity, edible, berry, 3–7 × 2.8–3.5 cm, bears tiny glochids

<b>Stem Anatomy</b>	: The shape of epidermis cells irregular or zigzag, anticlinal wall pattern; straight or slightly sinuate, length of epidermal cells noted $53.25 \pm 2.05 \mu\text{m}$ and width is $35.10 \pm 5.19 \mu\text{m}$ . The average epidermal cell number is 116 per unit area. The mean value of no. of stomata is 4 per unit area. Stomata paracytic, the stomatal length is $52.95 \pm 1.39 \mu\text{m}$ and width is $33.60 \pm 1.56 \mu\text{m}$ . The length of the subsidiary cell is $63.65 \pm 2.27 \mu\text{m}$ , and the width is $5.35 \pm 0.78 \mu\text{m}$ . The length and width of guard cells are $53.85 \pm 1.59 \mu\text{m}$ and $20.45 \pm 0.80 \mu\text{m}$ . The length of the stomatal pore is $24.30 \pm 1.23 \mu\text{m}$ , and the width is $9.60 \pm 0.55 \mu\text{m}$ . The Stomatal index is 3.23%. Trichomes absent
<b>Pollen Morphology</b>	: Pollen grain large, a polar, monad, pantoporate, psilate. The polar view shape of pollen is circular, while the shape in the equatorial view is the prolate spheroidal shape. The polar diameter is $112.08 \pm 3.35 \mu\text{m}$ was measured. The equatorial diameter was calculated $90(101.65 \pm 2.71)115 \mu\text{m}$ . The length and width of colpi were measured $22.10 \pm 0.95 \mu\text{m}$ and $13.22 \pm 1.32 \mu\text{m}$ , respectively. Exine thickness was noted $8.57 \pm 0.76 \mu\text{m}$ . The P/E ratio was found to be 1.10. No. of fertile and sterile pollen was calculated 78 and 23, respectively. The fertility of pollen was measured by 77.22%

### 3.2 Ethnobotanical Findings

In the present research work, only three species, *O. dillenii*, *O. ficus-indica*, and *O. monacantha* belong to the genus *Opuntia* was reported to evaluate ethnobotanically from Pakistan. Detailed ethnomedicinal uses of cactus *Opuntia* plants were described in Table 3.4 along with herbal folk recipes.

#### 3.2.1 Demographic Data of Informants

Forty peoples were interviewed at different localities of the research areas during field surveys to collect the ethnomedicinal data, their knowledge and experience about the cactus *Opuntia* through general conversations and semi-structured interviews. The field notes were documented on *Opuntia* species about their local names, parts used, administration route, and herbal recipes. Peoples were interviewed including folk peoples and local vendors. Mostly, the age range of informants was 41–55 years (55%), followed by 30–40 years (32%) and 55–76 years (13%). During the field survey, gender-wise males 85% and 15% were interviewed (Table 3.5). Commonly most of the informants were not highly educated.

#### 3.2.2 Cactus *Opuntia*

*Opuntia* Mill. genus has a remarkable number of pharmaceutical industry applications, food, and cosmetic goods abundantly (Stintzing and Carle, 2005). Conventionally in many countries of the world, the genus *Opuntia* species with their

**Table 3.4** Ethnobotanical and medicinal properties of Cactus *Opuntia* spp.

Sr. No	Plant specie	Local name	Life form	Part used	Preparation	Medicinal use and treatment of disease	Recipe
1	<i>Opuntia dillenii</i> (Ker-Gawl) Haw.	Thor, Nagphani, Ammar Phali, Zuqam	Shrub	Phylloclade, Stem latex, Stem bark and pulp of rudimentary leaves	Decoction, Juice	The latex produced by stem is effective for cattle eye disease, wound healing in the form of pasted through cloth on wound place is fixed to expel pus, also used against snakebites Juice of stem cladodes is useful in the reduction of body fever Stems cladodes along with spines positioned round the room after childbirth to prevent evil spirits (ghosts)	The paste in formed by mixing the inner fleshy part of stem cladodes with <i>Aloe vera</i> leaf and sugar is grounded for wound healing and utilized topically along with orally on affected places. Two teaspoons of this paste are taken once a day for 2-3 days early in the morning
			Leaves	Leaves	Oral, leafy juice	Leaf juice of permeate leaves is orally taken as a remedy for whooping cough, asthma, fever and burning sensation	Boiling of leaves in water. For 3 days one water cup is taken
			Fruit	Fruit	Raw food paste, syrup, juice	The fresh fruits are expectorant, edible and demulcent are taken directly as a tonic, also used for whooping cough after basking, for controlling spasmodic cough in syrup form, used for treatment of gonorrhoea. The juice of ripened fruits is remedially valuable for the treatment of asthma, gastric ulcer, diabetes, lowering cholesterol level and hepatic congestion	The prepared fruit extract with sugar put up in a bottle were used for 21 days, juice taken two times in a day of two tablespoons
			Flowers	Flowers	Paste	Abscess, eye disease	Both leaves and flowers crush together after removing spines and form decoction in the form of paste and apply it on that specific area

(continued)

Sr. No	Plant specie	Local name	Life form	Part used	Preparation	Medicinal use and treatment of disease	Recipe
2	<i>Opuntia monacantha</i> (Willd.) Haw.	Chithar thor, Nagphana, Thohar	Shrub	Whole plant	Juice, Powder	Used as carminative and stomachic, for healing the broken bones, strong the bones and for memory stimulant. Medicinally used for the urinary complaints, anemia, tumors, inflammation and ulcer. Plant juice was used for curing earache, ophthalmic and liver infection	Mingling the 1 <sup>1/2</sup> spoon of <i>Opuntia monacantha</i> with 1.5 spoon of <i>Cynodon dactylon</i> were taken with one glass of water, for 1 week or 1 month three times in a day depending on the ailment state
				Fruit	Raw form, Infusion	Eating ripened fruit for diabetes. Fruit mucilage used for treatment of syphilis and gonorrhoea. Mucilage mixture with turmeric is used as infusion for leprosy, strains, pox, rheumatism and piles externally	The fruit is taken three times daily in dried form to lowers blood glucose level
				Stem cladodes, Latex	Decoction, Poultice	Latex is rubbed on paralyzed organs are used against constipation. Stem ash functioned as cathartic and used as soothing poultice and used as a folk medicine for abdominal pain	10 honey drops along with 4-6 drops of latex is very effective
				Flower	Extract	Flowers extraction proved to cure respiratory, asthma and bronchitis	

Sr. No	Plant specie	Local name	Life form	Part used	Preparation	Medicinal use and treatment of disease	Recipe
3	<i>Opuntia ficus-indica</i> Mill.	Danda thohar, Thuar	Shrub	Leaves	Poultice	Dressing for boils	Heated with blue soap and Epsom salts and applied as a poultice
				Flower	Oral dosage	Medicinally used to treat disorders of calculus removal resulted in diuretic hustle, digestive system, cure ulcer, urological problems	Extract mixture of dry flowers is prepared
				Stem	Juice, Plaster, Decoction	Traditionally used as medicine for headache, diabetes, whooping cough, gastrointestinal disease, bacteriological infection, skin inflammation, diarrhea, kidney pains and piles	Boil in sesame oil and rub hair skin before wash, as direct application. Typical pastries prepared with juice of <i>Opuntia ficus indica</i> boiled and mixed with drench of wheat, almonds, hazelnuts, cinnamon and sugar
				Fruit	Powder, Raw	Also used as folk medicine for dyspnea, heals wounds of liver, fatigue, stomach pain and ulcer Fruit is used as foodstuff for humans and animals Daily fresh ten fruits are eaten	<i>O. ficus-indica</i> without seeds mixed with olive oil and garlic for the preparation of Sauce for pasta

**Table 3.5** Demographic data of informants about cactus *Opuntia* spp.

Sr. No.	Variables	Categories	No. of persons	Percentage
01	Informants	Local vendors	4	10
		Indigenous peoples	36	90
02	Gender	Male	34	85
		Female	6	15
03	Age	30–40 years	13	32.5
		41–55 years	23	57.5
		56–76 years	4	10
04	Educational background	Illiterate	31	77.5
		Completed 5 years education	4	10
		Completed 8 years education	2	5
		Completed 10 years education	2	5
		Completed 12 years education	1	2.5

stem segments (cladode) and edible fruit is being used nutritionally for human food (Pinto and Scio, 2014). The different parts of numerous *Opuntia* plants such as stem, fruit, flower, and the whole plant were described previously to be used in folk medicine and religious ceremony frequently (Stintzing and Carle, 2005; Arias and Véliz, 2006). It is also reported earlier that the cactus *Opuntia* has a wide range of biological activities such as analgesic, anti-diabetic, antimicrobial, hallucinogenic, anti-inflammatory, antioxidant, and anti-cancerous effects (Casado et al., 2008; Hahm et al., 2010; Castellar et al., 2012). These plants are rich in nutritional components (El Kossori et al., 1998; Hernández-Pérez et al., 2005) and valued as food colorants by the synthesis of pigments with the existence of parasites (Fernández-López et al., 2010; Castellar et al., 2012).

### 3.2.3 Plant Parts Used

The reported species of cactus *Opuntia* in the present research study were in shrubby life form. The most frequently part used in documented data were fruit (34%), followed by stem (25%), flowers (19%), rudimentary leaves (13%), and other parts (9%), as shown in Fig. 3.7. The fruits of cacti *Opuntia* are mostly used for ethnobotanical purposes. Fruits are the most frequently used part due to the presence of fruits on the top of the stem pads and containing many metabolites, nutrients and minerals. Their collection is easy compared to other parts like stem and flowers, which contain more spiny characters.

### 3.2.4 Mode of Administration

The well-known methods of herbal preparation from *Opuntia* plants were mostly in the form of raw material (27%) followed by paste (22%), juice (17%), and decoction (11%). While infusion (8%), extract (6%), powder (4%), and the remaining 5%

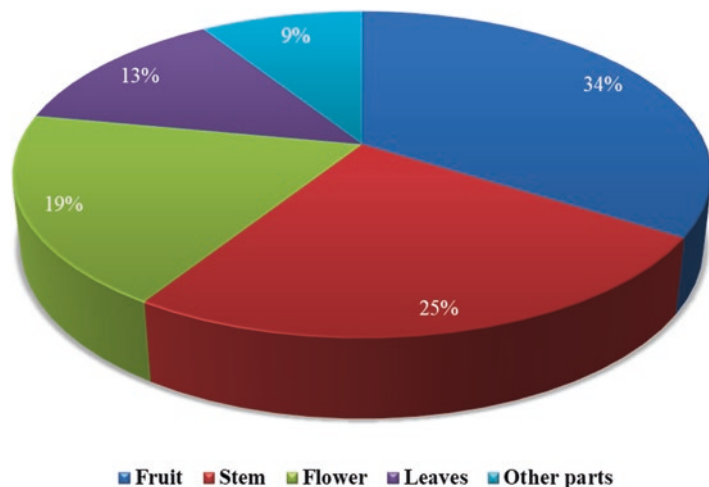


Fig. 3.7 Graph showing the percentage of different parts used of *Opuntia* spp.

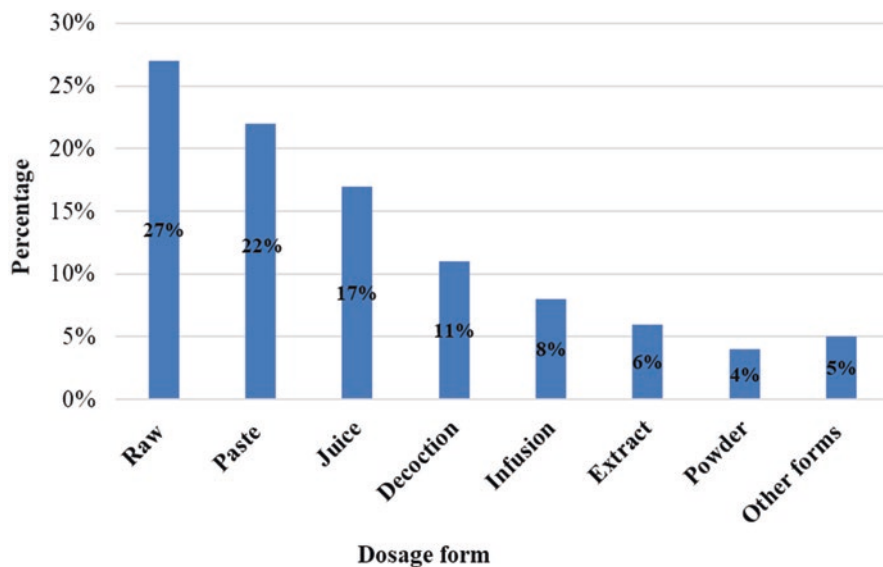


Fig. 3.8 Graphical representation showing mode of preparation form

are used in many other different forms. The administration route was mostly in the form of oral dosage 68%, followed by topical application (32%) (Fig. 3.8).



### 3.2.5 Quantitative Data Analysis

The quantitative ethnobotanical statistics of different relative indices, i.e., use value (UV), the relative frequency of citation (RFC), and informant consensus factor (ICF), were summarized in Tables 3.6 and 3.7.

#### (a) Use Value (UV) and Relative Frequency of Citation (RFC)

The UV values range from 0.14 to 0.5. The highest UV value was observed for *Opuntia dillenii* 0.5, followed by *Opuntia ficus-indica* 0.35, while the lowest UV value was recorded in *Opuntia monacantha* 0.14. The calculated range of RFC values were from 0.22 to 0.47. *Opuntia ficus-indica* showed the highest RFC value (0.47) followed by *Opuntia dillenii* (0.3), while the least value of RFC was noted in *Opuntia monacantha* (0.22).

#### (b) Informant Consensus Factor (ICF)

**Table 3.6** Quantitative values of UV and RFC indices

Sr. No	Plant specie	UR <sup>a</sup>	UV <sup>b</sup>	FC <sup>c</sup>	RFC <sup>d</sup>
01	<i>Opuntia dillenii</i>	7	0.5	12	0.3
02	<i>Opuntia ficus-indica</i>	5	0.35	19	0.47
03	<i>Opuntia monacantha</i>	2	0.14	9	0.22

<sup>a</sup>Number of use reports cited for species

<sup>b</sup>Used values

<sup>c</sup>Informants mentioning the use of plant species

<sup>d</sup>Relative frequency of citation

**Table 3.7** Value of ICF for disease treatment by *Opuntia* species

Sr No.	Category of disease	Nur <sup>a</sup>	Nur% <sup>b</sup>	Nt <sup>c</sup>	ICF <sup>d</sup>
1	Eye disease	3	6.97	2	0.5
2	Microbial infection	3	6.97	2	0.5
3	Diabetes	4	9.30	2	0.66
4	Hypertension	2	4.65	1	1
5	Respiratory disease	5	11.62	3	0.5
6	Gonorrhea infection	3	6.97	2	0.5
7	Blood circulatory disorders	3	6.97	2	0.5
8	Hepatic disorders	3	6.97	2	0.5
9	Digestive problems	6	13.95	3	1.5
10	Urological problems	3	6.97	2	0.5
11	Osteological problems	2	4.65	1	1
12	Gastrointestinal disorders	4	9.30	1	0.66
13	Renal disorders	3	6.97	1	1

<sup>a</sup>Number of use reports

<sup>b</sup>Percentage value use reports

<sup>c</sup>Number of species cited

<sup>d</sup>Informant Consensus Factor

The *Opuntia* plants utilization for specific disease treatment were reported. The 13 different disease categories were outlined including eye disease, respiratory disorders, diabetes, renal disorders, hypertension, microbial infection, blood circulatory disorders, urological problems, hepatic disease, gonorrhoea infection, osteological problems, urological problems, and gastrointestinal disorders. Digestive problems showed the highest ICF value of 1.5, followed by osteological, hypertension, and renal disorders 1, whereas the least value of 0.5 of ICF was observed for different categories of disease such as respiratory, microbial, and urological problems.

## 4 Conclusion

In this contemporary research, detailed information on ethnobotany, medicinal utilization, and the *Opuntia* species system was investigated in Pakistan. The observed taxonomic characters of *Opuntia* species, including (*Opuntia dillenii*, *Opuntia ficus-indica*, and *Opuntia monacantha*) was inspected. Although the basic morphological, anatomical, and pollen morphological features are similar, they also showed individual differences in some features that can be used as a tool to identify the species accurately. Different palyno-anatomical characters such as epidermal cell shapes, stomata type, guard cell shape, pollen type, exine thickness, ornamentation, and statistical data are reliable for characterizing the species. The reported ethnomedicinal documentation gives an effective outcome about the cactus *Opuntia*. The *Opuntia* species are highly medicinal for their usage as primary health care and herbal medications. It also has many applications in pharmaceuticals, and their uses has been of immense potential to be food in the future.

**Acknowledgment** We are also grateful to Higher Education Commission of Pakistan for funding under project No. NRPU-7837.

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**Part II**  
***Opuntia* Metabolism, Biodiversity, Species  
and Cultivars**



# Chapter 4

## Cactus Pear (*Opuntia* spp.) Species and Cultivars



Mouaad Amine Mazri

**Abstract** Cactus pear (*Opuntia* spp.) is a plant genus native to the American continent's tropical and subtropical regions. Today, cactus pear plants are cultivated in many parts of the world due to their high economic values, health-promoting benefits, and multiple uses. Indeed, cactus pear plays important socioeconomic, agronomic, and ecological roles. In many regions, the cactus pear is considered as a source of livelihood. It is used for human consumption since the fruit is delicious and rich in nutritive compounds such as amino acids, vitamins, proteins, minerals, dietary fibers, and phenolics. Cactus pear cladodes are used to feed livestock as they are rich in dietary fibers and characterized by high water retention capacity. Besides, the cactus pear is used in pharmaceutical industries as it contains bioactive compounds and has health promotive properties such as anticancer, anti-inflammatory, neuroprotective, and antioxidant activities. Besides, cactus pear plants can grow under different climatic and environmental conditions. The cactus pear is a crassulacean acid metabolism (CAM) plant genus that produces fruits and cladodes even under harsh conditions and water stress circumstances. It is known for its water use efficiency and has many ecological benefits such as degraded land rehabilitation, biodiversity preservation, and desertification prevention. The genus *Opuntia* comprises more than 300 species, the most economically important one among them is *Opuntia ficus indica*, which is used for human consumption and as forage. Other species such as *O. megacantha*, *O. amyclaea*, *O. streptacantha*, and *O. robusta* are widely cultivated for fruit production. The present chapter reports results from the literature regarding the characterization and diversity of cactus pear species and cultivars based on morphological, physicochemical, and molecular aspects. It highlights the phylogenetic relationships among cactus pear species and describes their main phenological events depending on the geographic area of cultivation. It also reports information regarding the ploidy levels of cactus pear species and interspecific hybridization experiments' main findings.

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**Keywords** Cactus pear · Phenology · Crassulacean acid metabolism · Morphology · Characterization · Cultivars · Species

## Abbreviations

CAM	Crassulacean acid metabolism
EST-SSR	Expressed sequence tag
GDH	Growing degree hour accumulation
ISSR	Inter-simple sequence repeat
ITS	Internal transcribed spacers
nuSSR	Nuclear microsatellite
RAPD	Random amplified polymorphic DNA
SSR	Simple sequence repeat

## 1 Introduction

*Opuntia* is a plant genus of the family Cactaceae widely cultivated throughout the world (Caruso et al., 2010). This genus comprises more than 300 species known as cactus pear, bunny ears, and prickly pear that cultivated for many purposes (Palevitch et al., 1993; Pinedo-Espinoza et al., 2017; Mazri, 2018). Cactus pear plays important socioeconomic, agronomic, and ecological roles since it is used for human consumption, as forage for cattle, for erosion control, to combat desertification, and in cosmetic and pharmaceutical industries (Nefzaoui and Ben Salem, 2002; Nefzaoui et al., 2014; Valadez-Moctezuma et al., 2015a, b, Mazri, 2018). Cactus pear fruits are consumed either fresh or after processing into different products such as juice, jam, puree, syrup, and gel (Sáenz, 2013a). Cactus pear fruits are rich in vitamins, minerals, proteins, carbohydrates, amino acids, phenolic compounds, and dietary fibers (Shetty et al., 2012). Cladodes have high dietary fiber, water, and ash contents (Mazri, 2018; Yahia and Sáenz, 2018). They are generally used to feed cattle, especially in arid and semi-arid regions, and as a vegetable in salads in some countries such as the USA and Mexico (Andrade-Montemayor et al., 2011; Costa et al., 2012; Mazri, 2018; Yahia and Sáenz, 2018). Cactus pear fruits and cladodes are also used in pharmaceutical industries since they contain bioactive compounds that have beneficial health effects because of their neuroprotective, hepatoprotective, antioxidant, antimicrobial, antiviral, anticancer, anti-inflammatory, and anti-diabetic properties (Benayad et al., 2014; Melgar et al., 2017; Mazri, 2018; Tilahun and Welegerima, 2018; Katanić et al., 2019). On the other hand, many cosmetic products are manufactured from cactus pear plants (Sáenz, 2013b).

Cactus pear is originated from the tropical and subtropical regions of America (Mazri, 2018). The archeological data showed that cactus pear plants were

cultivated and used by people in prehistoric Mesoamerica (Casas and Barbera, 2002). Cactus pear domestication started around 9600 years ago in the Mesoamerican region, where American natives selected and cultivated the species and genotypes that have the most advantageous characteristics (Casas and Barbera, 2002; Flannery, 1986). Indeed, cactus pear species were among the most important plant resources in Mesoamerica since they were abundant and could be used for human and animal consumption (Casas and Barbera, 2002). Cactus pear was introduced to Spain by the first European conquerors after discovering the American continent, and the first *Opuntia* plants were cultivated around Cadiz and Seville (Donkin, 1977; Ochoa and Barbera, 2017; Andreu-Coll et al., 2020). During the sixteenth century, cactus pear plants were introduced to many other European countries such as Italy, Germany, England, and the Netherlands, and subsequently to Australia (seventeenth century), China, India, and South Africa (eighteenth century) (Donkin, 1977; Zimmermann and Moran, 1991; Mondragón-Jacobo, 2001; Ochoa and Barbera, 2017). Today, Mexico is the world's largest producer of cactus pear fruits with 45% of the world production, followed by Italy (12.2%), which is also the world's largest exporter, and then by South Africa with 3.7% of the world production (Andreu-Coll et al., 2020).

Cactus pear species have developed anatomical, morphological, and physiological adaptations to different soil and environmental conditions. Accordingly, cactus pear plants are found today in many countries and can grow and develop even under harsh and difficult conditions where other plant species barely survive (Nefzaoui et al., 2014; Prat and Franck, 2017; Kumar et al., 2018). Cactus pear species also have high phenological flexibility that allows them to tolerate climate change impacts. The phenological events of cactus pear occur during different periods of the year, depending on the species and the climatic conditions of its geographic location (Reyes-Agüero et al., 2006).

Cactus pear plants may be spiny or spineless (Fig. 4.1). They have fleshy stems, also called pads or cladodes, containing spines or glochids arranged in areoles (Glimn-Lacy and Kaufman, 2006). The leaves are either small or absent since cladodes are involved in photosynthesis (Glimn-Lacy and Kaufman, 2006). The root system is extensive, close to the soil surface, and has xeromorphic characteristics



**Fig. 4.1** Spiny and spineless cactus pear plants

that allow the plants to survive to extended periods of drought (Glimn-Lacy and Kaufman, 2006; Prat and Franck, 2017). The flower is large, sessile, has several sepals and petals that appear as tepals, and a single pistil with a lobbed stigma at the apex (Glimn-Lacy and Kaufman, 2006; Arba et al., 2017). The fruit is an ovoid-spherical fleshy berry that may exhibit different colors at maturity (Fig. 4.2), consisting of skin, pulp, and seeds. The skin exhibits the same characteristics as the cladode and may have glochids and areoles (Beccaro et al., 2015; Mazri, 2018). The plant contains an outer surface cuticle that is thick and waxy, preventing water loss in drought conditions (Glimn-Lacy and Kaufman, 2006).

Cactus pear species are crassulacean acid metabolism (CAM). They are characterized by stomata closing during daytime and opening at night, a time during which cactus pear plants fix  $\text{CO}_2$  and accumulate and store malate in the vacuoles of the chlorenchyma cells (Inglese et al., 2017; Arba, 2020). This results in a less transpiration than  $\text{C}_3$  and  $\text{C}_4$  plants, and thus less water loss (Nobel, 1988). Besides, such characteristics allow cactus pear to have high water use efficiency and, as a result, high tolerance to drought conditions and superior adaptability to arid environments (Inglese et al., 2017).

Cactus pear is known for its taxonomic complexity (Valadez-Moctezuma et al., 2015a, b). This is due to the frequent and widespread interspecific hybridization (Wang et al., 1996, 1997; Griffith, 2001), the different ploidy levels of cactus pear species, and their conflicting phylogenetic positions (Martínez-González et al., 2020a). The taxonomic complexity of cactus pear highlights the complex genetic background of the species of this genus (Samah et al., 2016).

Cactus pear is characterized by high genetic diversity. Studies on the characterization of cactus pear species and cultivars were carried out in different countries. By employing different methods, the most frequently used among them are morphological descriptors and molecular markers (Bendhifi et al., 2013; El Kharrassi et al., 2017; Amani et al., 2019). While many authors attempted to discriminate cactus pear species and genotypes by using morphological traits, others suggested



**Fig. 4.2** Cactus pear fruits with different shapes and colors

considering molecular markers as they are not influenced by environmental conditions (Valadez-Moctezuma et al., 2015a). Physico-chemical studies were also conducted to characterize cactus pear fruits, juice, seeds, and cladodes (El Kharrassi et al., 2015; Núñez-Gastélum et al., 2018).

Among all cactus pear species, *Opuntia ficus indica* is the most economically important one (Palevitch et al., 1993). This spineless species is considered a domesticated form of *O. megacantha* and cultivated for fruit and cladode production (Labra et al., 2003). Other important cactus pear species cultivated for fruit production include *O. amyclaea*, *O. hyptiacantha*, *O. megacantha*, *O. robusta*, *O. streptacantha*, and *O. xoconostle* (Sáenz, 2013c; Yahia and Sáenz, 2018). Regarding cultivars, the most commercially important ones are found in Mexico and belong to *O. ficus indica*, and *O. megacantha* (Table 4.1).

The main results from the literature dealing with the phylogenetic positions, phenological events, and ploidy levels of different cactus pear species are reported in the present chapter. Besides, findings on the characterization of cactus pear species and cultivars from different countries by using morphological descriptors, molecular markers, and physicochemical traits are also reported.

## 2 Phylogenetic Characterization of Cactus Pear Species

The genus *Opuntia* is characterized by many species and genotypes and the high morphological similarity of many of them. This is due to the frequent and widespread interspecific hybridization and the different ploidy levels of the wild and cultivated cactus pear plants (Mazri, 2018). Accordingly, it has been challenging to establish a reliable and accurate classification of the genus *Opuntia* species. The advances made in the recent years in new generation sequencing technologies, as well as the growing body of publicly available sequence data, have helped in evaluating and describing the phylogenetic relationships among cactus pear species, and thus to have a clearer view on the origins of the genus *Opuntia* and to establish a more accurate classification of its species.

Majure et al. (2012) sequenced the plastid genes *matK* and *ycf1*, the plastid intergenic spacers *atpB-rbcL*, *ndhF-rpl32*, *psbJ-petA*, and *trnL-trnF*, the nuclear gene *ppc* as well as internal transcribed spacers (ITS) to evaluate the phylogenetic position of 98 species of the genus *Opuntia* and to reconstruct the phylogeny of the tribe Opuntieae. It was found that *Opuntia* s.s. is a well-supported clade that originated from southwestern South America then was expanded to different American regions. The clade had known a reticulate evolution and polyploidization, which resulted in a high morphological diversification, the production of new phenotypes, and a significant increase in the number of cactus pear species. Majure et al. (2014) assessed the phylogenetic relationship of three cactus pear species, *O. abjecta*, *O. triacantha*, and *O. militaris* by the plastid intergenic spacers *atpB-rbcL*, *ndhF-rpl32*, *psbJ-petA*, *trnL-F*, the plastid genes *ycf1* and *matK*, the nuclear ribosomal ITS and the nuclear gene *ppc*. These authors reported that *O. militaris* and *O. abjecta* were placed in

**Table 4.1** Most commercially important cactus pear cultivars

Specie	Cultivar	Geographic location	Fruit color	References
<i>O. ficus indica</i>	Rojo vigor	Mexico	Red	Astello-García et al. (2015), Mazri (2018), Ortega-Hernández et al. (2019)
<i>O. ficus indica</i>	Naranjona	Mexico	Orange	Carrillo-López et al. (2002), Mondragón-Jacobo and Bordelon (1996)
<i>O. ficus indica</i>	Roja Lisa	Mexico	Red	Mondragón-Jacobo and Bordelon (1996), Yahia and Mondragón-Jacobo (2011)
<i>O. ficus indica</i>	Rojo Pelón	Mexico	Red	Mazri (2018), Valadez-Moctezuma et al. (2015b)
<i>O. ficus indica</i>	Chicle	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. ficus indica</i>	Copena Torroja	Mexico	Red	Ochoa et al. (2015)
<i>O. ficus indica</i>	Algerian	South Africa	Pink	De Wit et al. (2010), Du Toit et al. (2018), Novoa et al. (2019)
<i>O. ficus indica</i>	Morado	South Africa	Green/white	De Wit et al. (2010), Du Toit et al. (2018), Novoa et al. (2019)
<i>O. ficus indica</i>	Gymno-Carpo	South Africa	Orange	De Wit et al. (2010), Du Toit et al. (2018)
<i>O. ficus indica</i>	Meyers	South Africa	Dark pink	De Wit et al. (2010)
<i>O. ficus indica</i>	Roedtan	South Africa	Orange	De Wit et al. (2010)
<i>O. ficus indica</i>	Van As	South Africa	White	De Wit et al. (2010)
<i>O. ficus indica</i>	Skinners court	South Africa	White/green	De Wit et al. (2010), Novoa et al. (2019)
<i>O. ficus indica</i>	Nudosa	South Africa	Red/ Orange	De Wit et al. (2010), Novoa et al. (2019)
<i>O. ficus indica</i>	Zastron	South Africa	White	De Wit et al. (2010), Novoa et al. (2019)
<i>O. ficus indica</i>	Rossa	Italy; South Africa	Red	Mazri (2018), Mondragón-Jacobo and Bordelon (1996), Novoa et al. (2019)
<i>O. ficus indica</i>	Bianca	Italy	White	Mazri (2018), Mondragón-Jacobo and Bordelon (1996)
<i>O. ficus indica</i>	Gialla	Italy; Tunisia	Yellow	Mazri (2018), Mondragón-Jacobo and Bordelon (1996)
<i>O. ficus indica</i>	Verde	Chile	Green	Mondragón-Jacobo and Bordelon (1996)
<i>O. ficus indica</i>	Blanca	Chile	White	Mondragón-Jacobo and Bordelon (1996)
<i>O. ficus indica</i>	Gigante	Brazil	Yellowish green	Edvan et al. (2020), Mazri (2018), Nunes et al. (2017)

(continued)

**Table 4.1** (continued)

Specie	Cultivar	Geographic location	Fruit color	References
<i>O. ficus indica</i>	Aissa	Morocco	Yellow to orange	Arba et al. (2002), Mazri (2018)
<i>O. ficus indica</i>	Moussa	Morocco	Yellow to orange	Arba et al. (2002), Mazri (2018)
<i>O. megacantha</i>	Rubi reina	Mexico	Red	Astello-García et al. (2015), Mazri (2018), Valadez-Moctezuma et al. (2015b)
<i>O. megacantha</i>	Amarillo Platano	Mexico	Orange	Ochoa et al. (2015)
<i>O. megacantha</i>	Huesuda	Mexico	Orange	Ochoa et al. (2015)
<i>O. megacantha</i>	Naranjon Legitimo	Mexico	Orange	Ochoa et al. (2015)
<i>O. megacantha</i>	Pico Chulo	Mexico	Orange	Gallegos-Vazquez (2006), Ochoa et al. (2015)
<i>O. megacantha</i>	Amarilla Monteza	Mexico	Yellow/orange	Gallegos-Vazquez (2006), Valadez-Moctezuma et al. (2015b)
<i>O. megacantha</i>	Camuezo	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. megacantha</i>	Roja Azteca	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. megacantha</i>	Roja San Martín	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. megacantha</i>	Rojo Lirio	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. megacantha</i>	Pico de Oro	Mexico	Orange	Valadez-Moctezuma et al. (2015b)
<i>O. megacantha</i>	Torroja	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. megacantha</i>	Rosa de Castilla	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. megacantha</i>	Achefri	Morocco	Greenish yellow	Mazri (2018), Sedki et al. (2013)
<i>O. albicarpa</i>	Esmeralda	Mexico	Light green	Gallegos-Vazquez (2006), Mondragón-Jacobo and Bordelon (1996)
<i>O. albicarpa</i>	Chapeada	Mexico	Light green/blush/Orange	Gallegos-Vazquez (2006), Mondragón-Jacobo and Bordelon (1996), Valadez-Moctezuma et al. (2015b)
<i>O. albicarpa</i>	Reyna	Mexico	Light green	Gallegos-Vazquez (2006), Mondragón-Jacobo and Bordelon (1996), Ochoa et al. (2015)
<i>O. albicarpa</i>	Rojo Ojuelos	Mexico	Red	Ochoa et al. (2015)
<i>O. albicarpa</i>	Blanca de Castilla	Mexico	Green	Valadez-Moctezuma et al. (2015b)

(continued)

**Table 4.1** (continued)

Specie	Cultivar	Geographic location	Fruit color	References
<i>O. albicarpa</i>	Bola de Masa	Mexico	Yellow	Valadez-Moctezuma et al. (2015b)
<i>O. albicarpa</i>	Copena Z1	Mexico	Green	Valadez-Moctezuma et al. (2015b)
<i>O. albicarpa</i>	Fafayuca	Mexico	Yellow/green	Gallegos-Vazquez (2006), Valadez-Moctezuma et al. (2015b)
<i>O. albicarpa</i>	Mango	Mexico	Yellow	Valadez-Moctezuma et al. (2015b)
<i>O. albicarpa</i>	Villanueva	Mexico	Green	Gallegos-Vazquez (2006), Valadez-Moctezuma et al. (2015b)
<i>O. albicarpa</i>	Reyna Crucen	Mexico	Green	Valadez-Moctezuma et al. (2015b)
<i>O. amyclaea</i>	Burrona	Mexico	Light green	Gallegos-Vazquez (2006), Ochoa et al. (2015)
<i>O. amyclaea</i>	Cristalina	Mexico	Light green	Mondragón-Jacobo and Bordelon (1996), Ochoa et al. (2015)
<i>O. streptacantha</i>	Cardona	Mexico	Red	Ochoa et al. (2015)
<i>O. streptacantha</i>	Charola Tardia	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. robusta</i>	Tapón Aguanoso	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. robusta</i>	Robusta	South Africa	Purple	Du Toit et al. (2018)
<i>O. undulata</i>	Oreja Elefante	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. undulata</i>	Bolañera	Mexico	Purple	Gallegos-Vazquez (2006)
<i>O. affinis</i>	Memelo	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. cochineria</i>	Cacalote	Mexico	Red	Valadez-Moctezuma et al. (2015b)
<i>O. joconostle</i>	Xoconostle Blanco	Mexico	Green	Valadez-Moctezuma et al. (2015b)

synonymy under the Caribbean species *O. triacantha*. They also assessed the relationship between the two species *O. cubensis* and *O. ochrocentra*, widely accepted as synonyms. The plastid and nuclear data showed that *O. abjecta* belongs to the Humifusa clade.

In contrast, *O. triacantha* belongs to the Nopalea clade and is closely related to *O. caracassana*, *O. jamaicensis*, and *O. guatemalensis*. Besides, *O. militaris* was found to belong to the Nopalea clade and is closely related to *O. caracassana*, but not to *O. triacantha*. On the other hand, the DNA sequence data suggested that *O. ochrocentra* is the result of hybridization between *O. abjecta* and *O. dillenii*, while *O. cubensis* s.str. is the result of hybridization between *O. militaris* and *O. dillenii*. Realini et al. (2015) evaluated the phylogenetic relationships of 15 cactus pear species from southern South American. The species studied were *O. penicilligera*, *O. quimilo*, *O. salmiana*, *O. anacantha*, *O. arechavaletae*, *O. aurantiaca*, *O. megapotamica*, *O. monacantha*, *O. bonaerensis*, *O. colubrina*, *O. discolor*, *O. elata*, *O. schickendantzii*, *O. ventanensis*, and *O. sulphurea*. The phylogenetic



relationships were examined by sequencing the plastid intergenic spacers *trnL-trnF* and *psbJ-petA*, as well as the ITS regions. The haplotype network and maximum likelihood analyses showed eight haplotypes for the Northern Hemisphere species, and 15 haplotypes for the southern South American species. Srikanth and Whang (2015) examined the phylogenetic position of three Korean *Opuntia* species, namely *O. ficus indica*, *O. humifusa*, and the new forma *O. humifusa* f. *jeollaensis* by analyzing the DNA sequences from the *trnL-F*, *atpB-rbcl*, *matK*, and ITS regions. The phylogenetic analysis showed that the Korean *O. humifusa* belongs to the Humifusa clade. The new forma *O. humifusa* f. *jeollaensis* was placed in the Macrocentra clade and was found to be closely related to *O. camanchica*. Surprisingly, *O. ficus indica* grown in Korea was closely related to *O. engelmannii* but not to the *O. ficus indica* sequences downloaded from the GenBank database. It was concluded that the Korean *O. ficus indica* is conspecific to *O. engelmannii*. Martínez-González et al. (2020a) attempted to provide a detailed morphological description of the species *O. joconostle* and establish a phylogenetic placement. *O. joconostle* was reported to have a high morphological similarity with *O. matudae* and considered a synonym of *O. ficus indica*. On the other hand, *O. matudae* was regarded as a synonym of *O. megacantha*. Accordingly, these authors used sequences from the ITS nuclear region and the *matK* and *trnL-F* plastid regions to evaluate these four cactus pear species' phylogenetic relationships. The findings showed that *O. joconostle*, *O. matudae*, *O. megacantha*, and *O. ficus indica* all have distinct phylogenetic positions and should be considered four different cactus pear species. In a different study, Martínez-González et al. (2020b) evaluated the phylogenetic position of *O. matudae* and *O. hyptiacantha* within the clade *Opuntia s.s.* Indeed, these two species have been widely believed to belong to the same taxon. The phylogenetic analysis was performed using sequences from the ITS nuclear region and from the *matK* and *trnL-F* plastid regions and highlighted that *O. matudae* and *O. hyptiacantha* are two different species and thus could not be included in the same taxon.

### 3 Ploidy Level of Cactus Pear Species

Cytogenetics may play a key role in establishing efficient breeding programs and the development of cultivars with improved characteristics. Besides, cytogenetic studies are highly useful to assess plants' genetic diversity, especially among the Cactaceae family (Ahumada et al., 2020). On the other hand, cytogenetics will be valuable in the implementation of a reliable classification of the genus *Opuntia*, which is complicated due to the different morphological features observed in the plants of this genus and that are caused by different factors such as the environmental conditions and the frequent and widespread occurrence of interspecific hybridization (Gallegos-Vázquez and Mondragón-Jacobo, 2011).

Cactus pear species have different ploidy levels, with a base chromosome number of  $x = 11$  (Mazri, 2018). According to Pimienta-Barrios (1994) and Mashope (2007), a high ploidy level (e.g.  $2n = 6x = 66$  and  $2n = 8x = 88$ ) is reflected in large

fruits and cladodes, and vigorous plants. Thus, the cultivated species and cultivars are generally hexaploid or octaploid, while the wild populations are generally diploid or tetraploid. The different ploidy levels observed in cactus pear species make them highly adaptable to different climatic and environmental conditions (Palomino et al., 2016).

Palomino and Heras (2001) reported that the three cactus pear species, *O. cochineria*, *O. hyptiacantha*, and *O. streptacantha*, collected from wild populations in Mexico, are all octoploids ( $2n = 8x = 88$ ). However, their chromosome size and genome length are different. *O. streptacantha* has the largest chromosomes and the longest genome. This was followed by *O. cochineria* then by *O. hyptiacantha*, which has the smallest chromosomes and genome length. Segura et al. (2007) employed flow cytometry to evaluate the genome size and ploidy level of 23 *Opuntia* species grown in Mexico and cultivated for edible fruit production. Four different ploidy levels were identified. The majority of species, including the most economically important ones such as *O. megacantha*, *O. ficus indica*, and *O. albicarpa*, were all found to be octoploid. At the same time, *O. heliabravoana* was the only diploid species. The other species were either tetraploid or hexaploid. Realini et al. (2014) evaluated the ploidy level of 14 *Opuntia* species from southern South America (Argentina, Uruguay, Bolivia, Brazil, and Paraguay). All the species exhibited the same chromosome base number ( $x = 11$ ), small-sized metacentric chromosomes, and symmetrical karyotypes. However, the ploidy level differed. For example, *O. schickendantzii*, *O. arechavaletae*, *O. anacantha*, *O. quimilo*, and *O. colubrina* have  $2n = 22$ ; while *O. discolor*, *O. penicilligera*, *O. bonaeriensis*, *O. elata*, *O. megapotamica* and *O. monacantha* have  $2n = 44$ . Palomino et al. (2016) determined the chromosome number and ploidy level of six cactus pear species from Mexico. Three different ploidy levels were detected: *O. heliabravoana* is diploid ( $2n = 2x = 22$ ), *O. jonocostle*, *O. matudae*, and *O. oligacantha* are hexaploids ( $2n = 6x = 66$ ), while *O. hyptiacantha* and *O. tomentosa* are octoploids ( $2n = 8x = 88$ ). Ahumada et al. (2020) examined the ploidy level of some of the widely cultivated cactus pear genotypes in Argentina, namely *O. ficus indica* (L.) f. *ficus-indica* (yellow tuna, cordobesa orange tuna, italiana orange tuna, and salteña tuna), *O. ficus indica* f. *amyclaea* (white tuna and reddish tuna) and *O. robusta* (cuaresma tuna). While all genotypes exhibited small and similar-sized symmetrical chromosomes, different ploidy levels were reported. *O. ficus indica* (L.) f. *ficus-indica* genotypes are all octoploids ( $2n = 88$ ), *O. ficus indica* f. *amyclaea* genotypes are hexaploids ( $2n = 66$ ), while *O. robusta* (cuaresma tuna) is tetraploid ( $2n = 44$ ).

## 4 Hybridization Between Cactus Pear Species and Cultivars

Under natural conditions, hybridization between *Opuntia* species is a common and widespread phenomenon, even between those with different ploidy levels (Griffith, 2001; Reyes-Agüero et al., 2006). Thus, interspecific hybridization may play a significant role in the genetic improvement of cactus pear. Studies on hybridization

between cactus pear species have been carried out by many researchers to evaluate the compatibility between *Opuntia* species and create new cultivars with superior characteristics. According to Mondragón-Jacobo and Bordelon (1996), cactus pear hybridization was first claimed at the beginning of the twentieth century by Luther Burbank and resulted in the production of spineless species. However, the works of Luther Burbank received criticism due to the lack of recognized publications and registrations (Mondragón-Jacobo and Bordelon, 1996). Since the 1990s, many interspecific hybridization trials were performed (Mazri, 2018). The following possible hybridizations were reported: *O. ficus indica* × *O. hyptiacantha*, *O. streptacantha* × *O. hyptiacantha*, *O. streptacantha* × *O. ficus indica*, *O. streptacantha* × *O. megacantha*, *O. aureispina* × *O. macrocentra* ‘azurea type,’ *O. Xspinosibacca* × *O. engelmannii* var. *engelmannii*, *O. macrocentra* var. *minor* × *O. macrocentra* ‘azurea type’, *O. macrocentra* var. *minor* × *O. macrocentra* var. *macrocentra*, and *O. lindheimerii* × *O. ficus indica* (Wang et al., 1996; Griffith, 2001; Felker et al., 2010). A review of literature by Reyes-Agüero et al. (2006) reported different possible hybridizations between cactus pear species based on cytogenetic, morphologic, artificial breeding and phytochemical evidences. The possible hybridizations reported were as follows: *O. chlorotica* × *O. phaeacantha*, *O. cochineria* × *O. leucotricha*, *O. cochineria* × *O. robusta*, *O. edwardsii* × *O. lindheimeri*, *O. edwardsii* × *O. phaeacantha*, *O. edwardsii* × *O. lindheimeri* × *O. phaeacantha*, *O. engelmannii* × *O. phaeacantha*, *O. grahamii* × *O. schottii*, *O. lindheimeri* × *O. phaeacantha*, *O. littoralis* × *O. megacantha*, *O. megacantha* × *O. phaeacantha* and *O. robusta* × *O. streptacantha* (Reyes-Agüero et al., 2006). According to Mondragón-Jacobo and Bordelon (1996), Mexican and South African cactus pear cultivars were developed based on hybridization and selection among wild and cultivated *Opuntia* species.

## 5 Phenological Characterization of Cactus Pear Species and Cultivars

The phenological events in cactus pear vary enormously depending on the species, cultivar, genotype/accession/ecotype, and the tree’s geographic location. For example, in the southern hemisphere, floral-bud development begins in September while it occurs in March in the northern hemisphere. Anthesis was observed between May and July in the USA and Europe. From February to August in Mexico, while fruiting was observed throughout the year, depending on the species and the geographic location (Reyes-Agüero et al., 2006). On the other hand, it is well known that the flowering and fruiting stages overlap in many cactus pear species. Osborn et al. (1988) compared the blooming periods of *O. polyacantha* and *O. phaeacantha* in southern Colorado (USA). They reported that the peak of blooming differs between these two cactus pear species. Blooming occurred first in *O. polyacantha* and lasted for a longer period. *O. polyacantha* bloomed in late May, and blooming lasted for 21–28 days while blooming occurred 7–11 days later in *O. phaeacantha* (5–11

June) and lasted for 16–25 days. In *O. maxima* and *O. stricta* from Catalonia (Spain), flowering and fruiting occurred between May and July (Gimeno and Vilà, 2002). El Kharrassi et al. (2015) investigated and compared the flowering and fruiting phenology of 2-year-old plants of six cactus pear species from different Morocco regions, namely *O. ficus indica*, *O. robusta*, *O. aequatorialis*, *O. dillenii*, *O. leucotricha*, and *O. stricta*. Phenological observations were performed based on the extended Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH)-scale (Hack et al., 1992) and The National Phenology Monitoring System (Thomas et al., 2010). The findings showed that vegetative bud emission starts in May's first week in all the evaluated species, except *O. robusta*. Flower buds were observed only in *O. ficus indica* and *O. dillenii* during the first week of May. These two species showed flowering during the second week of May while fruiting started in May's fourth week. The fruit growth took 4 weeks to reach the final size. The results reported by El Kharrassi et al. (2015) highlighted the fact that the occurrence of the phenological phases depends on cactus pear species and accessions. Segantini et al. (2010) monitored the phenological behavior of 4-year-old *O. ficus indica* plants from Brazil. Bud emission was observed between September and October, while flowering was observed 1 month after the flower buds' emission. The physiological maturity of fruits occurred 66 days after flowering. Arba et al. (2017) examined three cactus pear cultivars' phenological patterns belonging to two species grown in Morocco: *O. ficus indica* cvs. Moussa and Aissa, and *O. megacantha* cv. Achefri. It was found that the occurrence of the phenological events varies depending on the species, cultivar, and the year of study (2011 and 2012). For example, the emission period of vegetative and floral buds varied from 89 to 98 days in *O. ficus indica* cv. Aissa (started in January), 76–89 days in *O. ficus indica* cv. Moussa (started in February) and 76 days in *O. megacantha* cv. Achefri (started in February). Flowering started in March and ranged from 65 days in *O. megacantha* cv. Achefri to 89 days in *O. ficus indica* cv. Aissa, while fruit maturation started in June and lasted from 61 days in *O. megacantha* cv. Achefri to 84 days in *O. ficus indica* cv. Moussa. It was also reported that bud emission and flowering were mainly observed during spring, while summer corresponds to the period of fruit maturation. In *O. ficus indica* cv. Gialla (10-year-old trees) grown under Tunisian conditions, Aounallah et al. (2009) reported that bud transition from the vegetative state to the reproductive one occurs between March and the beginning of April. Besides, the asynchrony of flower differentiation was highlighted, since a group of flower buds was differentiated at the beginning of April, while a second group was differentiated in late April. The first group of flower buds required 13,620 growing degree hour accumulation (GDH) to reach anthesis, while the second group required 11,625 GDH. Based on microscopic and morphological observations, Aounallah et al. (2009) suggested six different growth stages from flower bud burst to fruit set: flower initiation, flower differentiation, growth in longitudinal diameters, pistil coloration, anthesis, and petal shedding. In *O. ficus indica* cv. Gialla grown in southern Sardinia (Italy), Nieddu and Spano (1992) reported that the flowering time was different among the different trees evaluated, and even within the same tree and cladode. Flower bud differentiation started in May, but the majority (76%) sets in June. The time between

flower bud emission and blooming ranged from 25 to 37 days, while that from blooming to fruit ripening ranged from 59 to 75 days.

Many factors influence the occurrence of the phenological stages of cactus pear species and cultivars. Arba et al. (2015) indicated that the climatic conditions and irrigation and fertilization practices all affect the flowering and fruiting phenology of cactus pear. According to these authors, in *O. ficus indica* cv. Moussa grown in Morocco, the emission of vegetative and floral buds and the formation of floral buds may start in February or March and lasts from 80 to 104 days, depending on the climatic conditions year of study, as well as on irrigation and the fertilizers used. Shoot emission lasts from 13 to 103 days (starting from April) depending on irrigation, fertilization, and the climatic conditions. The number of shoots developed also depended on the fertilizers used. On the other hand, it was reported that fertilization does not affect flowering (35–80 days, starting from March), fruit development (137–188 days, starting from February), and fruit maturation (59–111 days, starting from June).

The phenological stages of cactus pear species were reported to impact their biochemical characteristics. Pessoa et al. (2020) evaluated the nutritional properties of cactus pear cladodes depending on their phenological development. Five cactus pear varieties grown in Brazil and belonging to four species were compared: *O. ficus indica* (IPA-20), *O. ficus indica* (Gigante), *O. stricta* (Erect prickly pear), *O. undulata* (African prickly pear), and *O. atropes* (F-08). The cladodes were collected at three different phenological stages: young, intermediate, and mature. While there was no significant difference among the five cultivars and phenological stages regarding the concentrations of the dry matter, ether extract, and crude protein, other parameters such as mineral matter, pectin concentration, neutral detergent fiber, hemicellulose, acid detergent fiber, acid digested lignin and carbohydrates exhibited significant differences. It was shown that the Erect prickly pear and African prickly pear varieties have the best nutritional values and thus were suggested to feed ruminants.

## 6 Morphological Description of Cactus Pear Species and Cultivars

Cactus pear species and cultivars are characterized by great morphological variability (Mazri, 2018). It is well known that cactus pear plants can be spiny or spineless, the cladodes have different forms (e.g., round, elliptic, oblong), and the fruits exhibit differences in size, weight, shape, and color (e.g., white, green, yellow, orange, red, and purple). Accordingly, studies were carried out to describe the morphological aspects of both cultivated and wild cactus pear species and genotypes grown in different countries and regions (Mazri, 2018). Such studies have essential implications in the understanding of the botanical and taxonomic status of *Opuntia*, and to elucidate the complexity of this plant genus. The morphological descriptors that are

generally used to compare and characterize cactus pear species and cultivars are related to plant shape, cladode number, length, width, thickness and shape, presence and absence of spines, length of spines, length and color of flowers, shape, length, diameter of fruits, the weight of peel, and weight of flesh, as well as the number, size, and weight of seeds. These descriptors have a strong discriminating capacity.

On the other hand, different utilizations of cactus pear species and cultivars were suggested based on their morphological characteristics. For example, for human consumption either as fresh fruit or after processing into different products, or to feed cattle. The high morphological variability of cactus pear species and cultivars was also used for genetic breeding and conservation programs (Mazri, 2018; Amani et al., 2019). Many studies were carried out to evaluate the morphological diversity among the different *Opuntia* species, the genetic variability among cultivars was generally assessed among those belonging to *O. ficus indica*, since it is the most economically important *Opuntia* species.

Based on fruit and cladode morphology, Arba et al. (2002) identified and characterized the main cactus pear species and cultivars grown in Morocco. According to the observations of these authors, three species were distinguished: *O. schumannii* (spiny), *O. megacantha* (spiny), and *O. ficus indica* (spineless). Regarding *O. ficus indica*, four cultivars were morphologically described: Moussa, Aïssa, El Akria, and El Bayda. The cladode of these species varied considerably regarding their length, width, and thickness. The fruits also showed differences in color, length, and width, while there were little morphological differences among the four *O. ficus indica* cultivars. El Kharrassi et al. (2017) used ten different morphological descriptors to compare 124 cactus pear accessions from Morocco that belong to seven different species: *O. ficus indica*, *O. megacantha*, *O. robusta*, *O. aequatorialis*, *O. dillenii*, *O. leucotricha*, and *O. inermis*. The studied species showed important differences regarding the plant height and diameter, the average number of cladodes, the cladode width, thickness, the distance between areoles, and the average number of spines per areole (in spiny species). Three cactus pear species were spineless: *O. ficus indica*, *O. dillenii*, and *O. inermis*. Besides, the high similarity was observed between *O. ficus indica* and *O. megacantha*. In a different study, El Kharrassi et al. (2016) compared the fruit characteristics of 30 accessions of two cactus pear species (*O. ficus indica* and *O. megacantha*) from Morocco. While the mature fruits of *O. megacantha* accessions were all yellow-orange, those of *O. ficus indica* exhibited different colors: yellow-orange, purple-red, pink, and orange. The fruit length and weigh varied significantly depending on the species, accession, and geographic origin of the sampled plant. Hadjkouider et al. (2017) investigated the genetic diversity among five *Opuntia* species from Algeria, namely *O. ficus indica*, *O. amyclaea*, *O. streptacantha*, *O. engelmannii*, and *O. robusta*, by using 49 morphological and phenological descriptors. The morphological descriptors were related to the plants, cladodes, flowers, fruits, and seeds. Eight descriptors were found to be the most useful for cactus pear distinction. They thus were suggested for the classification of the species of the genus *Opuntia*: time of the beginning of flowering, time of harvest maturity, flower length, main spine color, the main color of fruit surface, color of fruit flesh, firmness of fruit flesh and length of the longest spine per cladode. It was

found that *O. ficus indica* and *O. amyclaea* are very close morphologically, but they are distinct from the other species evaluated. Núñez-Gastélum et al. (2018) assessed the variability of four wild *Opuntia* species (*O. engelmannii*, *O. polyacantha* var. *Arenaria*, *O. macrocentra*, and *O. phaeacantha*) from Mexico based on the characteristics of their seeds, which can be used as a novel source of functional food, oils, and nutraceuticals. The descriptors employed by these authors were seed weight, area, length, width, embryo and cotyledon area, and coat area. Significant differences were observed in the studied characteristics, with *O. polyacantha* var. *Arenaria* exhibiting the highest values in all the measured parameters. Amani et al. (2019) used 33 qualitative and 30 quantitative descriptors to assess the morphological variation among 48 ecotypes of cactus pear, belonging to four species (*O. ficus indica*, *O. engelmannii*, *O. tomentosa*, and *O. undulata*), all located in an *ex-situ* collection in Tunisia. The most discriminating traits were cladode shape, pulp color, fruit weight, fruit color, the number of seeds per fruit, the number of areolas per cladode, and the number of areolas at the cladode border. Besides, the high similarity between *O. ficus indica* and *O. engelmannii* was reported. Adli et al. (2019) assessed the morphological variability of 20 wild accessions of *O. ficus indica* using 30 quantitative and qualitative traits related to cladodes, areoles, and spines. The qualitative and quantitative analyses distinguished four major groups of accessions, mainly based on the presence or absence of spines and the number of spines per areole. Besides, it was suggested that some accessions were spineless but became spiny under stress conditions. Miguel et al. (2018) compared the morphology of two cultivars (Rossa and Gialla) and two wild ecotypes (Green and Orange) of *O. ficus indica* grown in southern Portugal. Significant morphological differences were observed in the fruits of the evaluated ecotypes and cultivars. The fruits of the wild ecotype ‘Orange’ showed the highest diameter and weight, while those of ecotype ‘Green’ showed the greatest length with the firmest flesh. Bendhifi et al. (2013) studied the genetic diversity of 28 cultivars of *O. ficus indica* originated from three regions of Tunisia, using various morphological descriptors related to the plant, cladodes, seeds, glochids, and fruits. Both quantitative (cladode length, fruit weight, and taste, pulp and peel weight, number of fruits per cladode, and number of seeds) and qualitative traits (fruit skin cracks, ground color and firmness, and internal pulp color) allowed cultivar discrimination and characterization, and were recommended for the identification of cactus pear cultivars.

## 7 Molecular Characterization of Cactus Pear Species and Cultivars

The genetic diversity among cactus pear species and cultivars has been widely assessed based on molecular markers. Molecular markers represent a powerful tool that allows detecting variability when it would not be possible with morphological descriptors, thus providing a stable classification of this plant genus’ species and

cultivars. Besides, molecular markers are not influenced by environmental conditions (Valadez-Moctezuma et al., 2015a; Mazri, 2018). According to Caruso et al. (2010), the use of molecular markers can reveal the close genetic relationship among genotypes that exhibit different phenotypic characteristics since such differences may be caused by somatic mutations that depend on the geographic region of cultivation. The use of molecular markers will help establish a reliable classification of cactus pear species and cultivars, which can be highly beneficial in breeding programs (Ganopoulos et al., 2015). Various molecular markers were used to investigate the genetic diversity and structure of cactus pear species and cultivars. The most widely used are random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), and simple sequence repeat (SSR) markers (Samah et al., 2016; El Kharrassi et al., 2017). Besides, cactus pear species and cultivars' genetic diversity was evaluated in different countries and under different climatic conditions. Similarly to the investigations carried out using morphological descriptors, the use of molecular markers to assess diversity within cactus pear cultivars was mainly performed in those of *O. ficus indica* (Bendhifi et al., 2013; Ganopoulos et al., 2015).

El Kharrassi et al. (2017) evaluated the genetic relationship among 22 ecotypes of seven cactus pear species (*O. ficus indica*; *O. megacantha*; *O. robusta*; *O. aequatorialis*; *O. dillenii*; *O. leucotricha*, and *O. inermis*) originated from different regions of Morocco by using 14 ISSR and 34 RAPD markers. The findings showed that 96.9–100% of the bands produced by the ISSR and RAPD primers were polymorphic. Simultaneously, a close genetic relationship between the two species *O. ficus indica* and *O. megacantha* was revealed. Samah et al. (2016) studied the most economically important Mexican *Opuntia* species' genetic diversity by using 13 SSR markers. The SSR loci showed a high polymorphism level. This study's overall findings suggested that the species *O. albicarpa*, *O. ficus indica*, *O. megacantha*, *O. hyptiacantha*, *O. lasiacantha*, and *O. streptacantha* do not have clear genetic boundaries. Rabeh et al. (2020) used five RAPD markers to distinguish eight *Opuntia* species growing in Egypt and have morphologically similar cladodes: *O. brasiliensis*, *O. dillenii*, *O. dejecta*, *O. ficus indica*, *O. tomentosa*, *O. phaeacantha*, *O. leucotricha*, and *O. microdasys*. The polymorphism level ranged from 70 to 89.4%, and the species were discriminated by the presence and number of unique fragments. The use of RAPD markers was thus suggested as an efficient means to identify and distinguish *Opuntia* species that exhibit high morphological similarity. Caruso et al. (2010) investigated the relationship among different cultivated *O. ficus indica* cultivars and compared them with other wild and cultivated *Opuntia* species. Sixty-two genotypes originated from different countries, and 16 *Opuntia* species were examined using six SSR and two expressed sequence tag (EST)-SSR markers. A high range of variation was observed in the SSR loci, which allowed to discriminate most of the genotypes evaluated.

Interestingly, some genotypes originated from different countries showed similar profiles. Besides, the cultivated cactus pear genotypes were clearly distinguished from the wild ones. Some cultivated cultivars of *O. ficus indica* showed close relationships to genotypes belonging to *O. albicarpa*, *O. megacantha*, *O. fuscicaulis*,



*O. streptacantha*, and *O. amyclaea*. Accordingly, Caruso et al. (2010) strongly recommended using molecular markers to reclassify cactus pear species and cultivars since the current classification, generally based on morphological traits, may be misleading. Valadez-Moctezuma et al. (2015a) used five RAPD and five ISSR markers to assess the genetic diversity of 52 cactus pear cultivars/accessions belonging to 12 different species. A 50.8% polymorphism was observed, and the findings showed that the clusters obtained after analyses do not reflect the current taxonomic classification. The authors suggested the existence of a smaller number of *Opuntia* species than what is currently known, and that the three species *O. ficus indica*, *O. albicarpa*, and *O. megacantha*, may have a common ancestry. Reis et al. (2018) evaluated the genetic relationships of 19 Portuguese cactus pear populations belonging to four species, namely *O. ficus indica*, *O. elata*, *O. dillenii*, and *O. robusta*. They compared them with the Italian *O. ficus indica* cultivars Bianca, Gialla, and Rossa. Fifteen nuclear microsatellite (nuSSR) markers were used and showed high genetic variability among the four species, with four major groups, each one corresponds to a distinct species.

In contrast, a low intraspecific variability was observed among the Portuguese populations of *O. ficus indica*. Bendhifi et al. (2013) screened ten RAPD markers to assess the genetic relationship and polymorphism of 28 *O. ficus indica* cultivars from Tunisia. Out of a total of 56 bands scored, 41 (73.2%) were polymorphic. The use of these markers helped in the discrimination and identification of Tunisian *O. ficus indica* cultivars. It was suggested as a tool to solve the homonymy problem within this species. Ganopoulos et al. (2015) used six ISSR markers to study the genetic variability within 22 Greek *O. ficus indica* accessions. A total of 57 bands were generated, exhibiting 50.2% level of polymorphism. The authors highlighted ISSR markers' reliability and their usefulness for genetic analysis, identification, and distinction of *O. ficus indica* accessions.

## 8 Physico-Chemical Characterization of Cactus Pear Species and Cultivars

The physicochemical properties of cactus pear fruits, seeds, and cladodes have been widely evaluated depending on the species, cultivar, and tree's geographic location. Such investigations are highly valuable for the valorization and efficient use of cactus pear species and cultivars (El Kharrassi et al., 2015). Plants of the genus *Opuntia* are characterized by high genetic variability and have been cultivated for many purposes, including human consumption, feed cattle, and pharmaceutical and cosmetic applications (Andrade-Montemayor et al., 2011; Mazri, 2018; Sáenz, 2013b). Accordingly, the physicochemical characterization of the fruits, seeds, and cladodes of cactus pear has been carried out by many researchers in order to identify the most accurate and appropriate utilization depending on species and cultivars.

Betancourt-Domínguez et al. (2006) evaluated the physicochemical composition of cladodes of different sizes from three commercial cactus pear cultivars, namely Blanco sin Espinas, Blanco con Espinas and Verde Valtierra, as well as a wild genotype from Mexico. Some physicochemical and nutritional parameters such as total soluble solids, water content, titratable acidity, and pH varied depending on the cladodes' size. On the other hand, the wild genotype exhibited interesting features comparable to those of the cultivated ones, if not better in some cases. El Kharrassi et al. (2015) compared the physicochemical properties of six cactus pear species in Morocco. The water content, ash content, total proteins, and total sugars of the cladodes of *O. ficus indica* (8 accessions), *O. robusta*, *O. aequatorialis*, *O. dillenii*, *O. leucotricha* (2 accessions), and *O. stricta* ranged from 86.6 to 92.0% (w/w), 12.9 to 22.1 g/100 g dry weight, 4.64 to 11.5 g/100 g dry weight, and from 3.22 to 12.5 g/100 g dry weight, respectively. These values varied significantly among the species and accessions evaluated. Gebresamuel and Gebre-Mariam (2012) extracted and analyzed the mucilage from cladodes of two cactus pear species, *O. ficus indica*, and *O. stricta*, grown in Northern Ethiopia. Significant differences were observed in terms of ash value and fat, moisture, and protein contents, while the crude fiber content did not differ significantly between these two species. Du Toit et al. (2018) determined the nutrient content of mucilage extracted from cladodes of four cactus pear cultivars: *O. ficus indica* cvs. Algerian, Morado, and Gymno-Carpo and *O. robusta* cv. Robusta. There was no significant difference in terms of total carbohydrates (62.1–62.8 g/100 g), starch (5.5–6.5 g/100 g), and ash (16.8–18.8 g/100 g), while significant differences were observed in some fatty acids (oleic, linoleic, arachidic,  $\alpha$ -linolenic, behenic and eicosatrienoic acids) and minerals (potassium and magnesium), with *O. ficus indica* cultivars having higher polyunsaturated lipid content and *O. robusta* cv. Robusta showing higher protein and monounsaturated lipid content. In *O. dillenii* from south India, Kalegowda et al. (2017) reported that the mucilage from mature cladodes is composed of 12% moisture and 4.7% ash; and that the main neutral sugar found in the mucilage of this cactus pear species is arabinose (38.8%), followed by galactose (33.0%) and then by rhamnose (15.7%).

El Kharrassi et al. (2016) analyzed the pH, total soluble solid content, titratable acidity, total carotenoids, vitamin C, and reducing sugars in the juice of 30 accessions of *O. ficus indica* (23 accessions) and *O. megacantha* (7 accessions) grown in Morocco. It was found that each accession has specific characteristics, regardless of the species and the geographic region from which the plants originated. The pH value, soluble solid content, titratable acidity, total carotenoids, and vitamin C content all significantly varied among species and accessions. The reducing sugar was the only component that did not differ significantly among the studied *Opuntia* species and accessions. Núñez-Gastélum et al. (2018) evaluated the chemical composition of seeds of four cactus pear species from Mexico: *O. engelmannii*, *O. polyacantha* var. *arenaria*, *O. macrocentra*, and *O. phaeacantha*. Significant differences were observed in terms of protein content, total phenolics, and some fatty acids. However, no significant difference was observed in total minerals and lipid content among the four *Opuntia* species evaluated. Miguel et al. (2018) compared the physicochemical parameters of fruits of two wild ecotypes (Green and Orange) and two cultivars

(Rossa and Gialla) of *O. ficus indica* grown in south Portugal. Significant differences were observed in total soluble solid content and titratable acidity, with cv. Gialla showing the highest °Brix value and the lowest titratable acidity percentage. The pulp of ecotype 'Green' showed the highest total fiber content, while glucose was the major carbohydrate found in all cultivars and ecotypes.

Regarding the organic acid content, malic acid was the only organic acid present in the peels and pulps of the four cultivars and ecotypes evaluated. Nadia et al. (2013) analyzed the physicochemical properties of the juicy pulp of four *O. ficus indica* cultivars (green, yellow, orange, and red) grown in north Algeria. Significant differences were found in pH, acidity, Brix percentage, protein content, ascorbic acid, total betalains, total carotenoids, and phenolic content. This study's findings highlighted the fact that the physicochemical properties of the same cactus pear species may vary depending on the cultivar. On the other hand, such characteristics may also differ depending on the geographic location of the tree. Indeed, according to Belviranlı et al. (2019), the physicochemical properties of the fruits and seeds of *O. ficus indica* trees grown in Turkey varied depending on their location. The °Brix value, crude cellulose, fructose, glucose, sucrose, raffinose, total phenolics,  $\beta$ -carotene, and ascorbic acid contents all showed significant differences depending on the geographic location of the tree. The mineral and phenolic contents also varied significantly, with potassium being the major mineral element in all fruit pulp samples. The effect of the tree's geographic location on seed oil content and some fatty acids (palmitic, oleic, linoleic, and erucic acids) was also revealed.

## 9 Conclusion

Cactus pear (*Opuntia* spp.) is one of the most important plant genus globally, especially in the arid and semi-arid regions. This is due to its phenological flexibility, high adaptability to harsh climatic and environmental conditions, and various uses of its fruits and cladodes. Despite the high importance of the genus *Opuntia*, its taxonomic classification is complicated due to different factors such as the lack of accurate and complete phylogenetic information and frequent and widespread inter-specific hybridization, and the different ploidy levels of cactus pear species. Thereby, many researchers claimed the necessity to reevaluate its current classification.

Characterization of cactus pear species and cultivars has been widely performed by using morphological descriptors. However, morphological traits can be influenced by environmental factors. Consequently, several investigations were carried out in recent years to compare and discriminate cactus pear species and cultivars by using molecular markers. On the other hand, many species and cultivars' physicochemical characterization was also performed to determine their most appropriate utilization.

To date, many cactus pear species remain underutilized. Thus, additional efforts are needed to characterize their fruits and cladodes to determine the best way to use them (e.g., human consumption, as forage, cosmetic and pharmaceutical industries).

It is also essential to develop new cultivars with high fruit quality (e.g., spineless, improved shelf-life) that are best suited for the international market. Much effort is also required to identify and characterize the best wild cactus pear populations.

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# Chapter 5

## Classification, Distribution and Morphological Characterization of *Opuntia* Species



Sidra Nisar Ahmed, Mushtaq Ahmad, Mohammad Zafar, Sofia Rashid, and Shazia Sultana

**Abstract** The genus *Opuntia* has its place in order Caryophyllales of Angiosperms. This group of evergreen, succulent perennial plants belongs to the family Cactaceae. The *Opuntia* genus is native to Central America, having approximately 181 known species. These species flourish in dry, warmer, and open areas. They are cultivated throughout the world as not only vegetable, crop, and food products like jams, juices, and beverages but used as fodder and forage in Brazil, Mexico, Northern and southern Africa, and in Western Asia. In the early stage, the cladodes are tender and used as a vegetable source known as Nopales, while its fruit is obovoid to spheroidal known as tunas. It is juicy and fleshy used in candies. They are considered alternative natural medicine in diabetes, colon cancer, obesity, gastric ulcers, and coronary heart diseases. The *Opuntia* species hybridize easily in the wild, leading to continuous morphological variations results in misclassifications. The present study aims to complete the morphological description (qualitative and quantitative) of *Opuntia* species characterized by flattened cladodes called pads, covered with areoles with tiny detached spines known as glochids. The flowers are mostly yellow, cup-shaped, and lack true petals. The species showed noteworthy differences among color, length, the diameter of cladodes, spines per areole, flower, and fruit shape and color. The *Opuntia* genus is one of the most ignored plants' genera, and this morphological characterization overcomes insufficient and inadequate knowledge for its species-level distinction.

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**Keywords** Morphology · Classification · Distribution · Stem · Spines · Fruit · Flower · Cladode

## 1 Classification of *Opuntia* species

*Opuntia*, the desert plant, is classified according to family structures with the help of a classification system that helps differentiate many cacti and classify them into groups. Robert Whittaker proposed a five-kingdom system of classification (Protista, Monera, Fungi, Plantae, and Animalia). Thus, all desert plants, including *Opuntia*, belong to the kingdom Plantae. *Opuntia* species contains vascular bundles (xylem and phloem), so they are placed under the phylum Tracheophyta. Their classification is based on plant structure, reproductive structures, and adaptive mode in the environment.

The genus *Opuntia* belongs to the family Cactaceae and order Caryophyllales. This order is classified under angiosperms-eudicots (Powell and Weedin, 2004). They are the ancestors of Rosids (angiosperm family) and after that the Asterids family (eudicot) (Holmes, 2016). The cactus *Opuntia* contains 127 genera and 1438 species. The arid-resistant plant *Opuntia ficus indica* (L.), generally called a cactus pear, having a vast range of properties, is cultivated widely throughout arid regions (Beccaro et al., 2015). It has a wide range of species, approximately 75–250. All these species of *Opuntia* are native to America. Among them, 181 species are known (Anderson, 2001), and approximately 76 wild species are found in Mexico (Orozco-Segovia et al., 2007). The cultivation of this “miracle plant” is increasing day by day as it is an essential source of fodder in deserts (Las Casas et al., 2017) as well as a source of food (Barbera, 1995; Ben Salem et al., 2000). They are perennial and succulent plants (having fleshy stem) of the family Cactaceae (Hunt et al., 2006). They are classified according to plant size and their pod’s size into three subgenera (Powell and Weedin, 2004). They are as follows:

1. **Cylindropuntia:** The *Cylindropuntia* have cylindrical stem segments but are arboreal or look like tree’s branches.
2. **Grusonia:** The *Grusonia* are short, prostrate (face down), and almost 30 cm high from soil having cylindrical stem pieces.
3. **Platyopuntia:** Its common name is “prickly pear” spherical/flat ring shape cladodes, also termed as pads. The *platyopuntia* have only one globular stem sections (pad).

The Cactaceae family contains significant new world plants widely spread in semiarid environments (Panda et al., 2017). It is further divided into four subfamilies.

### Subfamilies

1. Pereskioideae,
2. Maihuenioideae,
3. Cactoideae and
4. Opuntioideae.

Pereskioideae and Maihuenioideae comprise only one genus, while Cactoideae and Opuntioideae contain seven genus and two tribes correspondingly.

The subclass Opuntioideae has two tribes:

1. **Cylindropuntieae:** It comprises seven genera.
2. **Opuntieae:** It contains ten genera and 75 species.

The genus *Opuntia* ranked as the biggest group in Opuntioideae (Arakaki et al., 2011). Around 150–180 species under genus are recognized, and 66–83 of which are present only in Mexico (Hunt et al., 2006)

## 2 Distribution of *Opuntia* Species

The *Opuntia* species are well known for their ability to grow in dry and even desert conditions, and its cultivation has been especially important in marginal and subsistence economies (Stambouli-Essassi et al., 2015). Commercial plantations are concentrated in Spain, Italy, Mexico, Brazil, Chile, Argentina, and the USA (Basile, 2001). Prickly pear plants are used in various ways, such as food and beverage, feed, medicinal, and dyeing (Pimienta-Barrios et al., 2003). The *Opuntia ficus indica* (L.) Mill. commonly named prickly pear, is a long-domesticated cactus crop that is important in agricultural systems throughout arid and semiarid parts of the world (Anderson, 2001). It was introduced in the Mediterranean basin in the fifteenth century (Donkin, 1977). A wide range of varieties is cultivated worldwide. They are mainly differentiated by the plant habit and fruit characteristics (Perez et al., 1993). Some *Opuntia* species and cultivars are currently cultivated in several world regions in America, Africa, Asia, Europe, and Oceania (Felker and Inglese, 2003). The cactus cultivates in the wild, and the cultivated areas are at the height of 11–15 ft (3.6–4.8 m), including in the Mexican, Mediterranean, the Near East and North African areas, Israel, and Italy (Parera and Caloggero, 2004). It is a common fodder resistant to drought in Namibia (Tegegne, 2001) and rising in many freezing areas of the planet, including the southern US (Felker and Inglese, 2003).

The geographical region in which plants can be found is the geographical range (Yu et al., 2017) and is determined by various factors like climate, soil, and other plants' species. The coastal beaches of Southern Carolina fulfill these needs, and the atmosphere persists longer than the rest of the world (Szymanski and Zimbalist, 2006). The calcium soil is also very strong in the deserts of West Texas. The salinity of species that may grow in maritime grassland ecosystems is greater, reduced, or

stunted. This tends to make the ecosystem more accessible along with other factors such as dry land, colder weather, and nutrient status (Schenk and Jackson, 2002).

The *Opuntia* genus is used for big sweet fruits called tunas (Kamble et al., 2017). It constitutes a major weed problem for some areas of Australia, particularly in southeastern Queensland and some inland areas in Victoria and New South Wales (Melzer et al., 2000). The *Opuntia* species' kernel oil is extremely valuable due to fatty acids like linoleic acid (omega-3), cholesterol 9.5 g/kg, and vitamins (Ennouri et al., 2006).

### 3 Morphological Characterization of *Opuntia* Species

The genus *Opuntia* is important for many valuable purposes like medicine and in the treatment of several inflammatory diseases. The plants are grown in size from small plants to larger trees and mainly have fleshy and circular stems. The basic purpose of knowing about the morphology of *Opuntia* is to identify variations among different *Opuntia* species. There is a slight difference between the morphological variation of *Opuntia* as a low level of growth in wild species, and fruit-producing species has observed. The plant parameters such as size, color, shape, and symmetry depend on cultivation control. Many *Opuntia* species are described according to morphological characterization. A study-based data is collected in Kenya in which size, shape and color, and few other parameters are observed briefly. Many species have prolonged structures. For example, *Opuntia exaltata*, *Opuntia ficus-indica*, and 32 types of the population have an oval structure such as *Opuntia monacantha*, and *Opuntia stricta*. The stems arise most probably because they are smoothed, but fewer like *Opuntia exaltata* show ridges in stem structure (Omweri et al., 2016).

There is a variation in greenhouse grownup *Opuntia* plants as there are maximum cladodes thickness and maximum plant length compared to natural habitat *Opuntia* plants (Majure, 2007). These species have less need of soil and water, thus predicted to be the best from an agricultural point of view. The morphological characterization depends on length, width, plant size, stem shape, fruit pulp, peel fruit size, shape, and color.

The naturally complex xerophytes are species of *Opuntia* (Cactaceae, Opuntioideae). The genus is innate in America, and species are present in the southern ranges of South America (Powell and Weedin, 2004). In Mexico, where *Opuntia* species grow in arid environments due to their various xerophytic adaptations (DeFelice, 2004), the greatest diversity is found, containing condensed, waxy cuticles that minimize the extent of water lost by transpiration; adapted leaves and bud scales in the preparation of bristles and glochids (Mauseth, 2006), reducing the area of plants and transpiration rates and influencing temperature regulation (Lewis and Nobel, 1977; Nobel, 1978), fast root growth and superficial root systems that raise water absorption when rain interrupts the extended phases of drought.

*Platyopuntias* or genera of smooth stem *Opuntioidei cacti* (cladodes, or pads) are usually referred to as nopales or prickly pear cacti (Wallace and Fairbrothers, 1987). They can exist horizontally in order to straight and make small trees. They probably contain various fruits among several seeds, but they are still certainly reproduced from stem portions. Many species reorganize simply on the nodes, creating large clonal colonies via vegetative propagation (Rebman and Pinkava, 2001). Several pest and mite species (Mann, 1969) remain host to *Opuntia* species and are used by various animal species, such as humans (Kalmbacher, 1975; Majure and Ervin, 2008).

*Opuntia ficus indica* is gently a cactus cultivar and is essential in the agricultural system. In *Opuntia*, presence of cladodes is the chief fact of the plant. Variation among different *Opuntia* plants depends on genetic behavior and environmental conditions (Chalak et al., 2014).

## 4 Stem

The *Opuntia* species consist of chains called cladodes of flat stem parts (*platy opuntias*), the main photosynthetic site (Drezner, 2020). The stem consists of small portions and is stated as pads (Fig. 5.1). *Platyopuntia* pads are exposed as podaria and act as the important photosynthetic arrangement. *Opuntia* pads are enclosed in areoles, and their performance is similar to stomata and is areas for exchange for gases (Powell and Weedon, 2004). The *Opuntia* plant branches were identified (182 cm of tallness, 110 cm, and 137 cm in diameter).

In the genus *Opuntia*, the prickly-or cactus-pear plants are incorporated and are distinguished by the appearance of cladodes that are kind of combined stems as in



**Fig. 5.1** Pads of *Opuntia* species



**Fig. 5.2** Rounded cladode with hair like yellowish green glochids

Fig. 5.2. Their leaves frequently remain simple and temporary, or far away, and are presented by spines and pointed hair (glochids) borne by areoles (Stintzing and Carle, 2005). Its stem has different types of areole, having axillary buds that show spines are helpful concerning a new plant such as parts of the stem, flowers, etc. *Opuntia*'s areoles have different structures, such as rounded to elliptic, with color from white to gray. Its flower size ranges from 5 to 6 cm, and the flower color is from pink to red (Chauhan et al., 2010). Areoles are places where the spines are present, developing vegetative outgrowths and growing flowers. Several *Opuntia* species contain violet pads. *Opuntia* has its place in the *Caryophyllales*, therefore the color produced by the secondary complex of betalain present in their tissues. Betalains are nitrogen-comprise, water-soluble, vacuolar stains in their molecular organizations. They contain betacyanins and betaxanthins that produce red colors and yellow colors, respectively (Powell and Weedon, 2004).

The *Opuntia* stem has many segments, and it also contains betacyanin dyes that impart color to its flower, pads, and spines. Subsequently, the interruption of chlorophyll, secondary stains give the impression. Perennial plants organize this for next year's development as a way of keeping nutrients. Betalain pigments are supposed to help in drought and cold resistance (Ramakrishna and Ravishankar, 2011). Using age, stem parts stay green and are enclosed with 3 in. long bristles. Several areoles contain very small bristles. The larger bristles are very painful, but very small spines give rise to many discomforts if they are stuck in clothing or attached to the skin exterior (Gilman, 1999).

Plants of *Opuntia* grow delicious stems known as pads, vegetables, pencas, cladodes, or nopales. The kind of undeveloped portion of the cactus stem, new cladode or nopalitos, is mostly eaten up in salads as a vegetable. However, the cactus pear fruit is ingested as fresh fruit (Díaz et al. 2017).

## 5 Cladode

Though the funnel-shaped leaves of *Opuntia* are understood in the spring, the leaves have several millimeters in length with no venation. Prickly pear cactus has ordinary leaves; still, the stem's plate-like segments are frequently considered leaves. These plate-like segments are altered stems in actually having a length of almost 2–6 in. (Gilman, 1999). *Opuntia ficus-indica* seems to be the most commonly consumed cactus only for the processing of fruits and silage. A cladode can develop 35–40 flowers; each would reduce up to 3 g of water on day time, relative to 15% of its weight at the point of reproductive phase, during extensive flowering in times of rapid evapotranspiration rate (De la Barrera and Nobel, 2004; Inglese, 2010). Dependent on the microclimate, cladode sizes are highly variable, varying from 3.1 to 8.5 (–17.7) cm in length, to 2.0–5.2 (–9.0) cm in width and 4–10 (–19) mm in thickness. They may be in the form of ovoid, oblong, orbicular, or pear-shaped. Cladodes, often from the similar plant, can be strongly varied in morphology (Lucas C Majure and Ervin, 2008).

## 6 Spines

There are very limited *Opuntia* species that are spineless. Maximum spineless arrangements are cultivars of species which, contain spines. Spines (Fig. 5.3) are a modification for herbivorous defense, light safety and often help in reproduction, as various animals speedily displace and come to be tangled in animal fur and carry away as asexual clones. Glochids, which are piercing trichomes countless millimeters in measurement, are more frequently go along with spines. These are



Fig. 5.3 Leaf spines with areoles of *Opuntia* species



furthermore modification to herbivores but can also function as a characteristic for water preservation. To avoid rapid evaporation, a borderline is also made close to the surface of leaves or pads and helps to hold the air still. The glochids are pale orange, brown, or glowing more frequently, and exposure to ambient effects darkens them (for instance, sunlight). Glochids vary in extent up to 6 mm, but adaptable sizes of glochids are shown in a similar areole, where elongated inner glochids typically give way to a smaller outer circle of glochids.

The spines of *Opuntia humifusa* are yellowish or cream and flapped, typically in circles at the site of the spine when undeveloped, with red and reasonable browns; they turn a pale to bright white color with yellowish edges. Aging spines have yellowish, hazel, or dark tips, which turn bright or black-gray. They typically range from 5 to 71 mm, emerge at the basis 0.7–1.3 mm in diameter, and are firmly retrospectively bristles at juvenile stage, exhausting bristles throughout maturity level. Some species per areole has roughly two spines; however, some are spineless (Lucas C Majure and Ervin, 2008). The spines in *Opuntia ficus indica* are presented in Fig. 5.3.

## 7 Flowers

The *Opuntia* species mainly takes the most beautiful flowers globally, using their coloration owing to betalain complexes. Blossom time commonly occurs subsequently April to June, based on the state that inhabits the species. The bee-pollinated, eatable flowers are widespread hermaphroditic flowers, which means they are monocious (having male and female reproductive structures). In *Opuntia humifusa*, the central tepals are almost lemon-yellow. There are olive green external tepals, including bright colored edges. White is the style and the stigma. Lemon-yellow or milky shades are the filaments of stamens. Anthers are yellow (Lucas C Majure and Ervin, 2008). In *Opuntia* species, the few flowers only blossom for 1–3 days, while others flowers blossom for 6 or 7 h. This is an alteration to water preservation for waterless areas in which they inhabit. On the superior portion of the cladode, flowers are innate to more than 25, and maximum (90–95%) are modified into fruits. Flowers are usually large and present independently on the areoles. The perianth is designed as calyx with petaloid sepals and a corolla using sepaloid petals. Comprising 3–20 cyclical carpels, the ovary is deduced, and stamens vary in numbers (Arba et al., 2017).

## 8 Fruit

The *Opuntia* is a complicated genus that comprises species called “nopalitos” generally collected with *Opuntia ficus-indica*, such as for their fruits (from several species) recognized as cactus pears (tunas in Spanish) and *Xoconostles*, being used a

small edible cladode (Madera-Santana et al., 2018). The *Opuntia* species has significant possibilities to remain the diet of the future (Shetty et al., 2012) and primarily used worldwide for the processing of fruit (Amani et al., 2019; Nobel et al., 1992; Russell and Felker, 1987). The cactus pears, known by their fragrant, juicy, and sordid endocarp, stay morphologically dissimilar from their partners (Gallegos-Vázquez et al., 2012). Its fruit size ranges from 19.5 to 132 g, fruit pulp percentage is 50–60%, and 120 g of fresh fruit weight is essential (Reis et al., 2017).

The fruits of *Opuntia* are chiefly fleshy, desiccated, ovoid, spheroidal, pear-shaped growing for fall and in September. However, most fruits contain glochids and bristles, while certain fruits are smooth. Seed coats of *Opuntia* are rigid and constraining chemicals; these chemicals prevent sprouting under harsh conditions. They are comprised of wide-ranging colors like white, lemon-yellow, violet, red, and orange established by betalains. These consist of extended berries having a mass of about 67–216 g (Moßhammer et al., 2006). Betalains are of two types, betaxanthins and betacyanin yellow/orange and red/violet respectively, these create a fascinating palette of ordinary coloring agents (Melgar et al., 2017).

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# Chapter 6

## Cultivation and Cultural Practices of *Opuntia* spp.



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**Abstract** The genus *Opuntia*, family Cactaceae, originated in Mexico and spread to arid and semiarid regions worldwide. *Opuntia ficus-indica* (L.) Mill. is one of the most agro-economically important cactus species due to its fruit value with high nutritional and medicinal importance. Fruit can be consumed fresh, processed, or as a juice. Some *Opuntia* species are wild, and fruit is not suitable for human nutrition, but plants can be used as forage for animal nutrition. Moreover, *Opuntia* spp. is used to produce cladodes that are widely used as a type of vegetables. Cultivation of *Opuntia* spp. is an easy process. To achieve good results, several factors should be considered, such as the location, planting method, environmental conditions, soil type, cultural practices, and harvest stage to produce high yield with good quality.

**Keywords** Cactus pear · Prickly pear · Temperature · Irrigation · Drought · Salinity · Fertilization · Pruning · Flowering · Cropping · Fruit quality · Cladodes · Forage

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## Abbreviations

BAP	6-Benzylaminopurine
CAM	Crassulacean acid metabolism
DM	Dry matter
EC	Electrical conductivity
FDP	Fruit development period
IBA	Indole-3-butyric acid
Kc	Crop factor
PAR	Photosynthetically active radiation
PPF	Photosynthetic photon flux
PPFD	Photosynthetic photon flux density
PVC	Polyvinyl chloride
RFI	Re-flowering index
RH	Relative humidity
RUE	Rainfall use efficiency
SAI	Stem area index
SD	Saturation deficit
SF	Spring flush
TSS	Total soluble solids
WUE	Water use efficiency

## 1 Introduction

Originated in Mexico, *Opuntia* spp. (family Cactaceae, commonly called cactus pear or prickly pear), are one of the most important fruit plants that successfully cultivated in many regions of the world characterized by high temperature, drought conditions, erratic rainfall, and poor soils (Silva and Acevedo, 1985; Corrêia, 1986; Timbau, 1987). The name *Opuntia* came from an ancient Greek village in the region of Leocrid, Beocia: *Opus* or *Opuntia*, where Tournefort found a spiny plant that reminded him of the American opuntias in 1700. The genus *Opuntia* descended from Caryophyllales order, Cactaceae family, Opuntioideae subfamily, Opuntieae tribe. *Opuntia* genus includes 11 subgenera; *Opuntia*, *Consolea*, *Austrocylindropuntia*, *Brasiliopuntia*, *Corynopuntia*, *Cylindropuntia*, *Grusonia*, *Marenopuntia*, *Nopalea*, *Stenopuntia* and *Tephrocactus* (Scheinvar, 1995). There are almost 300 genus *Opuntia* species (Scheinvar, 1995), with almost 104 species and varieties recorded in Mexico alone (Bravo-Hollis, 1978). *Opuntia* quickly spread to Spain and grow as a wild or cultivated plant throughout different countries in the Mediterranean basin of the arid and semi-arid geographical zones (Velázquez, 1998). *Opuntia* are commonly polyploidy and show high variable phenotypes according to the environmental conditions. Reproduction is either sexually or asexually, and there are several interspecific hybrids (Swart et al., 2003). *Opuntia ficus-indica* (L.) Mill., is the most

important economic cactus species worldwide (Kiesling, 1999; Abo-El-Ela et al., 2001).

In 1988, a worldwide expansion of cactus pear cultivation was predicted, based on the plant's particular environmental biology, to develop sustainable agricultural production systems in areas with limited water and poor soils (Inglese et al., 1995). *Opuntia* distribution and productivity vary considerably among species, according to the environmental conditions. It is essential to know the region's physiography, defined in terms of rainfall (García de Cortázar and Nobel, 1991). Interest has recently increased to *Opuntia* to combat desertification, especially with the low cost of production and high economic return. *Opuntia* plants are likely to play an essential role in sustainable agricultural systems of many arid and semi-arid regions. Cactus pear is mostly known as a fruit crop, and it is slowly achieving the status of the formal crop in world statistics. Estimated data indicated that it is present on a commercial scale in over 20 countries, covering a planted area of around 150,000 ha (Mondragón-Jacobo and de Gallegos, 2017). The fruit has high nutritional and medicinal importance and consumed fresh, processed, or juice. They can also be used to produce cladodes that are used as a type of vegetables. Some *Opuntia* species are wild, and fruit is not suitable for human nutrition, but the entire plant can be used as forage for animal nutrition (Reynolds and Arias, 2001).

From an agronomic viewpoint, to rehabilitate or improve rangeland, shrubland, bushland, or poor farming areas characterized by shallow, stony, steep, or sandy soil or where the climate is too dry for practical farming, an appropriate planting strategy must be adopted. For instance, a plant density of 1000–2000 single or double cladodes per ha, with a spacing of 5–7 m between rows and 1–2 m within rows can be applied. In general, no special treatments (e.g., fertilizer application, pruning, and treatment against pests and diseases) are applied. Supplemental irrigation may be applied during establishment, but only if the first year is too dry. Plantations are exploitable after 3–4 years and fully grown after 7–10 years; if rationally managed, they can remain productive for >50 years. The productivity of rangeland planted with cactus can be increased by a factor of between 1 and 10 when very degraded and between 1 and 5 when in good conditions (Le Houerou, 2002).

There was an increased interest in cactus pear fruit production due to a greater demand for exotic fruit in the world's markets (Inglese et al., 1995). The plant is cultivated in a wide range of environments, resulting in differences in orchard practices, fruit quality, and crop productivity (Inglese et al., 2009; Liguori and Inglese, 2015). By the 1990s, much more information has become available on orchard management and other cultivation aspects of cactus pear (Felker and Inglese, 2003), which improve plant production and make it one of the most major crops in the twenty-first century. Griffiths (1909) and Turpin and Gill (1928) ascribed that cactus pear does not need to be cultivated like other crops, and just some little or no care still gives high productivity in virtually any environment. However, this perception has been rejected, and in order for the cactus pear to be profitable, the plant requires appropriate care for good fruit yield and quality (Potgieter and D'Aquino, 2017).

As a succulent and drought-tolerant plant, it can produce >20 t ha<sup>-1</sup> year<sup>-1</sup> of dry matter (DM) and provide 180 t ha<sup>-1</sup> year<sup>-1</sup> of water stored in its cladodes,

representing a cost-effective option for livestock watering (Dubeux et al., 2015). At such productivity levels, it is possible to produce sufficient forage to sustain five adult cows per year, which approximately represents at least a 60-fold increase over rangeland productivity. With small, intensively cropped cactus orchards, it is possible to produce feed and reduce the pressure on overstocked rangelands. Major cactus species used as forage include *Opuntia ficus-indica* Mill. and *Nopalea cochenillifera* Salm-Dyck., with several varieties released in different countries. Cactus cladodes from pruned fruit orchards can also be used as livestock feed, complementing farmers' income. In general, the forage potential of cactus in semi-arid areas is underutilized. There are tremendous opportunities to develop cactus-based livestock production systems, promoting human livelihood and reducing the pressure on natural rangelands (Dubeux et al., 2017).

Originated and evolved in central Mexico and known by Mexican consumers as nopalitos, the utilization of tender cladodes is being increased worldwide. Farmers are utilizing the vegetative structure, which is renewed by the continuous harvest. They exploit pruning's physiological effects, securing valuable greens year-round, in a region dominated by a climatic bimodal cycle of wet and dry seasons. The introduction of vegetable nopalitos in other countries and cultures has not been easy, despite the plant's adaptability and high productivity in most locations. The presence of thorns, even in the so-called "spineless" cultivars that present spines when young; the abundance of mucilage; and the lack of organoleptic appeal of cooked nopalitos have limited their adoption. Small orchards of nopalitos are found in the United States of America, mainly in California, Texas, and Arizona, and produce limited quantities intended for local farmers' markets, aiming at consumers of Hispanic origin (Mondragón-Jacobo and de Gallegos, 2017).

The cultivation of *Opuntia* spp. is an easy process. To achieve good results, several factors should be considered, such as the location, planting method, environmental conditions, soil type, cultural practices, and harvest stage to produce a high yield with good quality.

## 2 Plant Materials

### 2.1 Seeds

Seeds are not the common method of propagation due to genetic segregation in sexual reproduction; instead, they are being used in breeding programs. *Opuntia* breeding started in Brazil in 1985, with 85 clones obtained from seeds derived from open pollination of cv. Palma Redonda, plus 17 other clones from several Brazilian locations. The seeds are extracted from ripe fruit, then washed, sun-dried, and disinfection with diluted (10%) commercial bleach before planting. Germination is enhanced by scarification, i.e., seed immersion in hot water at 80 °C, followed by cooling off at room temperature, and then overnight soaking. Seeds can be planted



in standard germination media at high temperature (30–35 °C) and regular irrigation for good germination rates (Mondragón-Jacobo, 1999). Germination starts after a week but can continue for up to 2 months, depending on seed condition, cultivar, and species. Once the first cladode attains 5–10 cm, it can be transplanted to small plastic bags or pots and placed in a nursery. Providing long-day illumination and fertigation can accelerate plant growth in the nursery. Plants with a second cladode (25–40 cm height) are mature enough to tolerate field conditions, but they are still sensitive to frosts. Therefore, they should be planted after any waves of chilling temperatures are over (Mondragón-Jacobo et al., 2001). *Opuntia* flowers are also capable of self-pollination, and bagged flowers can set fruit and produce seeds that can be used as an asexual method of propagation, maintaining the same characteristics of the mother plant (Nerd and Mizrahi, 1994).

## 2.2 Cladodes

The cladodes are the common vegetative reproductive method of *Opuntia*, which is preferred by growers due to its simplicity (Mondragón-Jacobo and Pimienta-Barrios, 1995). They can initiate the rooting process soon after being in contact with soil, developing roots, branches, or flowers (Boke, 1980; Sudzuki Hills, 1995). Soil moisture is an important but not limiting factor for rooting because the cladode's stored water supports root initials. The source for planting material should be 1–4 years old, collected from healthy and vigorous plants. After separation from the mother plant at the joint with a sharp knife, the base disinfected with Bordeaux mixture (1 kg copper sulphate, 1 kg calcium hydroxide, and 100 L water) and allowed to air-dry in the shade for 4–6 weeks, so they can undergo a healing and suberization process to seal the potential sites of water loss and the entry of the immature stages of cochineal insect (Potgieter, 2001; Inglese et al., 2002a). Cladode bruising should be avoided to increase transplanting success (Lopez-Garcia et al., 2001). If stored in a shaded location, the cladode can sustain water loss for almost 6 months without losing viability, and they can be remaining alive for at least 12 months (Nobel and Castañeda, 1998). Arba (2009) reported that 1- or 2-year single cladodes could be used, although 1-year cladodes develop more and longer roots than 2-year cladodes. In general, single, mature, large, terminal cladodes of uniform appearance, visually free of any defects, insects, and diseases, should be selected as good plant materials (Potgieter, 2007). The cladode size does not affect its ability to form shoots or roots (Mondragón-Jacobo and Pimienta-Barrios, 1995), but it is positively correlated with the number and size of the new roots and shoots. Barbera et al. (1993b) and Wessels et al. (1997) reported that the surface area and dry mass of a cutting significantly influence successful rooting and budding in the field. A surface area of 500 cm<sup>2</sup> or a dry mass of 70–100 g allows good plant growth. Single cladodes as propagation units have the advantage of lower transport costs and easier handling during the planting process (Potgieter and D'Aquino, 2017). Cactus pear can also be propagated using multiple mature attached (one, two, or

more) cladodes. This is a common practice where 2–3-year multiple cladodes are used (Tudisca et al., 2015). Most of the basal cladodes are placed underground to ensure plant stability (Inglese, 1995). Multiple cladodes allow more rapid plant development and earlier fruiting than single cladodes (Homrani Bakali, 2013; Nasr, 2015). However, due to their size and mass, they are more challenging to handle and transport (Potgieter and D’Aquino, 2017). Under greenhouse conditions, new cladodes’ growth is markedly influenced by the basal cladodes’ dry weight, which acts as a carbon source for the new shoots (Luo and Nobel, 1993). One whole cladode can produce at least one layer of new shoots in a year, based on the cultivar and soil moisture content during the growing season. Healthy and vigorous branches with two or three pads are the best choice to start a new farm. *Opuntia* have an advantage over other fruit crops used in extensive and low maintenance plantations to avoid desertification (Fabbri et al., 1996).

### 2.3 Micropropagation

This method of vegetative reproduction is used with some *Opuntia* spp. such as *O. ellisiana*, used as fodder due to the low availability of planting stock. The plant was multiplied from explants containing areolas using *in vitro* culture techniques (Juárez and Passera, 1998). The sterilization procedure that showed the best results (only 12% areolas infected) consisted of immersing the entire cladodes in sodium hypochlorite plus Tween 80 and then in benzalkonium chloride solution. Explants were cultivated in Murashige and Skoog culture medium, supplemented with sucrose and different indole-3-butyric acid (IBA) and 6-benzylaminopurine (BAP) concentrations, at 27 °C, 100% relative humidity (RH), and a 16-h photoperiod. Explants in the medium containing 2.25 mg/L BAP and 2.0 mg/L IBA showed 100% shoot development in 35 days of culture. The mean shoot length was 10.2 mm after 49 days. A 100% root induction in shoots was obtained in a medium with 5 mg/L IBA after 12 days of culture. The highest numbers of roots were obtained when the entire shoots were cultivated in a medium supplemented with 5 and 10 mg/L IBA after 48 days of culture. The *in vitro* regenerated plants’ acclimatization was accomplished in a greenhouse, and plants showed good performance when transferred to soil (Guevara and Esteves, 2001).

## 3 Site Selection

The worldwide distribution of cactus pear indicates its extensive adaptability to various climatic and soil factors (Brutsch, 1979).

### 3.1 Climatic Factors

#### 3.1.1 Temperature

Temperature not only influences metabolic processes, and hence daily net CO<sub>2</sub> uptake, but extreme temperature can also lead to injury and even death of the plant. In general, *Opuntia* plants are incredibly tolerant to high air temperature, but not to air temperatures substantially below zero. When plants are acclimatized to high day/night air temperatures of 50 °C/40 °C, their chlorenchyma cells are not seriously injured by 60 °C for 1 h, and most cells can survive for 1 h at 65 °C (Nobel, 1988). High-temperature damages are only observed near the soil surface, where soil temperature in the desert can reach 70 °C. The young plants or newly planted cladodes are especially vulnerable to such injury. On the other hand, cell injury in the field occurs at freezing temperatures of -5 to -10 °C, which varies with the cultivar (Russell and Felker, 1987). The freezing damages increase with the reduction of the temperature, the duration of freezing conditions (Nobel, 1988), and with the stem water content, as lower water content leads to better tolerance to cold temperature (Cui and Nobel, 1994a; Nobel et al., 1995). Certain wild *Opuntia* species, such as *O. fragilis* (Nutt.) Haworth and *O. humifusa* (Rafinesque), both native to Canada, can tolerate temperatures below -20 °C when properly acclimatized (Nobel and Loik, 1993).

Because CO<sub>2</sub> uptake for crassulacean acid metabolism (CAM) plants (e.g., *Opuntia*) occurs primarily at night, the temperature at night is more important than that of the daytime for daily net CO<sub>2</sub> uptake. The optimal night temperature is relatively about 15 °C and temperatures from 5 to 20 °C lead to at least 80% of the maximal net CO<sub>2</sub> uptake. Such low temperatures also lead to low rates of transpiration. Net CO<sub>2</sub> uptake decreases as the temperature increases due to stomatal closure. Stomata are only one-third open at 20 °C and closed at 30 °C (Nobel and Hartsock, 1984). Except for night-time temperatures below zero or above 30 °C, the temperature is generally not a major limiting factor for net CO<sub>2</sub> uptake, especially in seasons when water from rainfall is available.

#### 3.1.2 Light

Another environmental parameter affecting net CO<sub>2</sub> uptake is light. Light incident on the individual stem can be manipulated by plant spacing, although trade-offs occur between maximizing net CO<sub>2</sub> per plant versus net CO<sub>2</sub> uptake per unit ground area (García de Cortázar and Nobel, 1991). The stem of the plant is opaque, contrary to the case for the leaves of most C<sub>3</sub> and C<sub>4</sub> plants. Therefore, both sides' orientation must be considered when evaluating light absorption. The main light wavelengths absorbed are referred to as the photosynthetic photon flux (PPF) = 400–700 nm wavelength, which is also referred to as the photosynthetic photon flux density (PPFD) and the photosynthetically active radiation (PAR)

(Nobel, 1999). Under dark conditions, only respiration occurs with a slight loss of CO<sub>2</sub>. As the daily PPF increases, the daily net CO<sub>2</sub> uptake increases. The plant reaches light saturation at a total daily PPF of about 25 mol m<sup>-2</sup> day<sup>-1</sup> (Nobel, 1988). The opaque nature of the stem makes some surfaces are not favorably oriented to the sunlight, and the interplant shading also reduces daily net CO<sub>2</sub> uptake, leading to a reduction in the plant productivity per unit ground area (García de Cortázar and Nobel, 1991). When the total stem area ratio to the ground area, termed as the stem area index (SAI), is 1, 2, and 3, the net CO<sub>2</sub> per unit ground area is 35%, 62%, and 85%, respectively (Nobel, 1991).

### 3.1.3 Atmospheric CO<sub>2</sub>

The current atmospheric CO<sub>2</sub> level on Earth is 413.40 ppm, and it is annually increasing by about 2.27 ppm by volume (Earth's CO<sub>2</sub>, 2020), which can lead to an increase in daily net CO<sub>2</sub> uptake by *Opuntia* plants. Cui et al. (1993) reported that a doubling of the atmospheric CO<sub>2</sub> level (360 ppm in 1993) caused net CO<sub>2</sub> uptake by 2-month-old cladodes to increase by 49%, and their water use efficiency (WUE) to increase by 55%. Nobel and Israel (1994) reported that total DM productivity was 37–40% higher for a doubled CO<sub>2</sub> level (Nobel and Israel, 1994). Although the nitrogen (N) content of older cladodes remains near 1% of the dry mass, the N content of 3-month-old cladodes averages 1.47% of the DM at atmospheric CO<sub>2</sub> level of 362 ppm, but 1.26% at doubled atmospheric CO<sub>2</sub> levels. The lower N content at the higher atmospheric CO<sub>2</sub> level reflects the lower amount of photosynthetic enzyme activities (Cui and Nobel, 1994b). Under wet conditions and moderate temperatures, net CO<sub>2</sub> uptake becomes positive in the late afternoon. Generally, a small burst of net CO<sub>2</sub> uptake occurs at dawn, when the availability of light allows for the direct incorporation of atmospheric CO<sub>2</sub> into carbohydrates using the C<sub>3</sub> pathway of photosynthesis during the coolest part of the daytime. The daily pattern of water vapor loss *via* transpiration is similar to the net CO<sub>2</sub> uptake pattern, reflecting the requirement of stomatal opening for gas exchange purposes. The CO<sub>2</sub> taken up by a CAM plant at night is bound to a three-carbon compound to form a four-carbon organic acid, such as malate, which stored overnight in large vacuoles within the cells of the chlorenchyma tissue (the greenish chlorophyll-containing region), so the tissue becomes more acidic during the night. The amount of CO<sub>2</sub> released from the organic acids during the next daytime resulted in reduced tissue acidity. This released CO<sub>2</sub>, which is prevented from leaving a CAM plant by daytime stomatal closure, is then incorporated into photosynthetic products in the chlorenchyma cells in the presence of light. The daily acidity oscillation, which is the characteristic of CAM plants, requires large vacuoles for the sequestering and short-term storage of the organic acids (Nobel, 2001).

### 3.1.4 Relative Humidity (RH)

Like most of the species having a CAM carboxylation pathway, *Opuntia* spp. require high RH. *Opuntia ficus-indica* is eliminated from areas where the average RH remains below 40% for more than 1 month consecutively (Le Houérou, 1994). The humidity saturation deficit (SD) is also essential for cacti. Le Houérou et al. (1993) reported that cacti normally grow in the arid and semi-arid zones of North Africa and the Near East, as mean annual RH and SD in these areas are usually above 60% and below 12 HPa, respectively. For that reason, cacti cannot grow in the African Sahel, where the average annual RH is 38%, and the mean SD is 24 HPa; nor do they grow in the Sudanian zone with RH of 52% and SD of 17 Hpa. They normally grow well in Eastern and Southern Africa with annual RH of 60–65% and SD of 10–12 HPa; in the Sahelian Atlantic shores where RH is 66% and SD is 11 HPa.

### 3.1.5 Rainfall

The absolute minimum requirement for rain-fed cultivation is a long-term mean of about 200 mm annually, provided soils are sandy and deep, as in the North Africa region. The plant can grow in zones with annual precipitation of 200–250 mm, but commercial production limits are around 450 mm year<sup>-1</sup> (Pimienta-Barrios, 1990). On silty and loamy soils, the minimum requisite is 300–400 mm annual precipitation. Cacti generally could be cultivated with some additional irrigation to reach a minimum total soil intake of 300–400 mm annually. Drainage is the most crucial ecological requirement for *Opuntia* since the plant is susceptible to the lack of oxygen in the root zone and cannot withstand any prolonged water logging. Thus, clay soils that may be temporarily saturated, poorly drained, or waterlogged is avoidable for cacti cultivation under sub-humid and humid Mediterranean climates, where annual rainfall exceeds 600–800 mm. The optimal soils for plant growth and development, in terms of aridity, is the semi-arid zone with a mean annual rainfall of 400–600 mm, as in cacti' homeland in the Mexican highlands, as well as areas with particularly on steep slopes (Le Houérou, 1981; Granados and Ana Dunia Castañeda, 1991; Pimienta-Barrios, 1994). In southwest Asia, the minimum mean annual rainfall is about 300–350 mm, because of the strict winter rainfall regime; hence the very long and absolute summer drought that lasts for almost 4 months in the semi-arid zone and over 6 months in the arid zone are not favorable conditions for *Opuntia* (Le Houérou, 1982).

## 3.2 Edaphic Factors

### 3.2.1 Water Relations

Besides using CAM with its inherently high WUE, *Opuntia* spp. have other adaptations that lead to water conservation. For instance, the waxy cuticle on its stems is relatively thick, generally 5–30  $\mu\text{m}$  (North et al., 1995). This helps prevent water loss from the plant to the surrounding environment. Besides, the stomatal frequency is usually low for *Opuntia*, generally 20–30 per square millimeter (Pimienta-Barrios et al., 1992). Consequently, the fraction of the surface area of the stem through which water vapor can move from the plant to the atmosphere is relatively low. Moreover, the stem contains a large volume of whitish water-storage parenchyma, which acts as a water reservoir for the chlorenchyma, where the initial CAM  $\text{CO}_2$  fixation and the daytime photosynthesis take place. Goldstein et al. (1991) reported that a drought period of 3 months decreased the thickness of the collenchyma cells by 13%, while the water-storage parenchyma decreased in thickness by 50%, indicating a greater water loss from the latter tissue. Roots also tend to be shallow with mean depths near 15 cm, facilitating a quick response to light rainfall. Kausch (1965) stated that *Opuntia* form new roots within 24 h of wetting dry soil. Its various water-conserving strategies lead to a small root system, where roots compose only about 12% of the total plant biomass (Nobel, 1988). Drought decreases the plant's ability to uptake  $\text{CO}_2$  from the atmosphere. Little change in net  $\text{CO}_2$  uptake occurs during the first week of drought, reflecting water storage in the stem and the low water requirements for CAM plants. Also, the waxy cuticle and low stomatal frequency allow 20% of the maximal net  $\text{CO}_2$  uptake to be present even 1 month after the plants are under severe drought. Thus, the net  $\text{CO}_2$  uptake ability of *Opuntia* spp. and some other CAM plants is extremely well suited to the arid and semi-arid regions. Nevertheless, soil water is the major limiting factor for net  $\text{CO}_2$  uptake in such regions, where irrigation may not be economically feasible. A useful benefit: the cost index for gas exchange by plants is the ratio of  $\text{CO}_2$  fixed by photosynthesis to water lost by transpiration, referred to as WUE. The WUE is about triple found for highly productive  $\text{C}_4$  plants (e.g., maize or sugar cane) under similar environmental conditions. High WUE for CAM plants is mainly related to the reduced difference in water vapor concentration between the plant and the atmosphere during the period of substantial stomatal opening. In particular, the water vapor content in leaves and stems is within 1% of the air's saturation value at a given tissue temperature (Nobel, 1999). Because tissue temperature typically averages at least 10  $^\circ\text{C}$  lower at night than during the daytime in many locations, CAM plants tend to lose only 20–35% as much water as  $\text{C}_3$  or  $\text{C}_4$  plants for a given degree stomatal opening. This is a key feature in *Opuntia* spp. utilization as a forage crop in arid and semi-arid regions (Nobel, 2001).

### 3.2.2 Nutrient Relations

Net CO<sub>2</sub> uptake, growth, and productivity are influenced by the content of macronutrients and micronutrients in the soil and salinity and soil texture (Hatzmann et al., 1991). Cacti perform well in deep, light-textured soils, including coarse sands, but clayey soils are not preferable. Shallow soils tend to give low yields. Cacti are tolerant of pH up to 8.5, and maximum electrical conductivity (EC) at soil saturation should not exceed 5–6 mS cm<sup>-1</sup> (Le Houerou, 1992). Growth in sandy loam soils is about 25, 50, and 75% at 0.03, 0.07, and 0.15% N of the soil DM, respectively (Nobel, 1989a). Since the N content in native sandy soils in arid and semi-arid regions is generally below 0.07%, the N fertilization usually increases plant growth in such areas (Nobel et al., 1987). The N fertilization protocol of *Opuntia* spp. has followed traditional practices developed for other crops (Barbera et al. 1992a, b; Nerd et al. 1993a, b), where the main N form is taken up from the soil is nitrate (Nerd and Nobel, 1995). The plant contains about 1% N of the DM in poor soils, whereas this percentage increases to about 2% of periodically fertilized soils (Nobel, 1988). Although N is generally the major growth-limiting nutrient, the growth of *Opuntia* is usually is stimulated by phosphorus (P) and potassium (K) fertilization (Nobel, 1989b). A P level of only 5 ppm of the soil DM improved growth by 50% (Nobel, 1989b). Most *Opuntia* spp. are sensitive to soil salinity. Inhibition of growth is often linear with sodium (Na) content, with 150 ppm leading to approximately 50% inhibition of biomass accumulation (Nobel, 1989b). The roots are more affected by salinity than the vegetative part of the plant. Berry and Nobel (1985) found that irrigation with 60 mM sodium chloride (NaCl) (about 12% of the seawater salinity) for 6 months reduces root growth by 84% and shoot growth by 50% (Berry and Nobel, 1985). Plants exposed to 100 mM NaCl for 10 weeks showed a reduction of 38% in root growth (Nerd et al., 1991a, b). The growth of a single root has reduced by 93% after only 4 weeks at the same NaCl concentration (Gersani et al., 1993). In general, sodium is not readily transferred from roots to shoot, or from basal cladodes to new cladodes (Berry and Nobel, 1985). Cactus cultivation can maintain soil organic carbon and carbon recalcitrance index at a similar level to the forest soil, contributing to the sustainability of the agro-ecosystem and climate change mitigation. Therefore, it could comprise a promising viable option for sustainable crop production systems under temperate sub-humid conditions, promoting socio-economic development and rural growth (Bautista-Cruz et al., 2018).

## 4 Cultivation

Thorough planning is crucial when establishing a commercial *Opuntia* orchard, and many important decisions must be made before planting. Many aspects require consideration, such as microclimate of the growing site, physical and chemical soil analysis, cultivar selection, soil preparation, ordering of plant material, planting of

windbreaks, determination of planting distances and row orientation, and installation of an irrigation system (Potgieter and D'Aquino, 2017).

#### 4.1 Cultivar Selection

The available number of cactus pear fruit cultivars varies significantly from one country to another. Mexico and South Africa have numerous cultivars, while in most other producing countries, the cultivar choice is rather limited (Inglese et al., 2002a). The choice of a fresh-fruit cultivar depends primarily on the planting site's climatic conditions and the market demand. *Opuntia* species and cultivars differ greatly concerning their potential fruit yield, quality characteristics, and adaptability to environmental conditions. Not all cultivars are equally adapted to a particular area; indeed, most cultivars exhibit strong genotype-environment interaction (Potgieter, 2007). Cultivars must be chosen wisely because changing the cultivar after orchard establishment entails very high costs. Besides, cultivar characteristics must be carefully considered before a final decision is made; specific cultivar preferences may be preferred for supplying a particular market (Potgieter and D'Aquino, 2017). For example, cultivars with a red, pink, orange, or yellow pulp are preferred on most European and North American markets, whereas white and green pulp fruit is favored by South African (Wessels, 1988c) and Mexican consumers (Mondragon-Jacobo and Perez-Gonzalez, 1994, 1996), as well as the Middle East and North Africa region. If growers are contemplating exporting fruit, the cultivar choice becomes even more critical as characteristics such as appearance, postharvest resistance to handling, and shelf life play a significant role in successful export. Producers should consider that some cultivar characteristics can be influenced by the environment or by orchard management practices. For example, although fruit mass is mainly genetically controlled, it can be influenced by fruit thinning, irrigation, fertilization, and pruning. Annual variation in fruit yield and quality is also evident in most commercial orchards. Some common varieties are; Algerian, Meyers, Morado, Nudosa, and Turpin from South Africa; Reyna, Cristalina, Burrona, Naranjona and Roja Vigor from Mexico; Gialla, Rossa, and Bianca from Italy; Amarilla sin Espinas from Argentina; Verde from Chile; and Andy Boy from USA (Potgieter and D'Aquino, 2017).

Varieties indicated for forage production and adapted to the specific environmental conditions should be used, and a variety of trials need to be carried out in different locations and regions to select high-performing ones. It is essential to consider the prevalence of pests and diseases in some areas and choose the region's best variety. In Brazil, for example, common varieties include 'Gigante' and 'Redonda', *Opuntia ficus-indica* Mill., but an outbreak of cochineal, *Dactylopius opuntiae* Cockerell, led to a change in the variety recommendation. Producers in the region are currently planting varieties tolerant to this insect, such as 'Orelha de elefante Mexicana', *Opuntia* spp. and 'Miuda', *Nopalea cochenillifera* Salm-Dyck (Dubeux et al., 2017).



In contrast to the wide range of cultivars available for fruit or forage production, the commercial vegetable (nopalito) cultivars are limited to ‘Milpa Alta’, ‘Atlixco’, and ‘Copena V1’, which originated in Mexico and belonged to *Opuntia ficus-indica* (L.) Mill. Other lesser-known varieties useful for nopalito production in tropical semi-arid areas are Valtierrilla, Blanco, and Spinless 1308. These varieties are spineless that can be fully utilized, providing tender cladodes, fruits, and sometimes mature pads for domestic animals (Mondragón-Jacobo and de Gallegos, 2017).

## 4.2 Soil Preparation

Pre-plant soil preparation is essential for successful cactus production and cannot be adequately performed after orchard establishment. Land clearing and leveling may be required (Inglese, 1995); irrigation supply lines may be installed, and plant rows marked out. In areas where domestic animals can damage young plants, fencing is required. Adverse soil conditions, such as impenetrable layers, perennial weed infestation, and shallow soil depth, need to be addressed before planting (Wessels, 1988b; Inglese et al., 2002a). Deep soil cultivation (at least 50 cm) with a ripper/subsoiler on the plant row is needed to break up any hardpan layers and improve drainage, aeration, and water-holding capacity. In heavier compacted soils, deep cultivation across the rows may also be beneficial because it helps the plant to survive during low rainfall years when it can utilize soil water from deeper soil depths (Potgieter, 2001). On very shallow soils, ridging is recommended (Singh, 2003). It is important to remove perennial weeds before establishment—either mechanically or chemically—as they compete strongly with cacti plants, particularly during the early stages of plant growth (Wessels, 1988b).

Soil amendments to correct soil nutrient imbalances and soil pH must be carried out before orchard establishment. A soil pH of 6.5–7.5 is considered optimum (Wessels, 1988a, b; Singh, 2003). Pre-plant fertilization should be based on soil analysis results, which indicate the level of plant nutrients in the soil. The topsoil’s representative soil samples (0–30 cm) and subsoil (30–60 cm) are needed for chemical and physical analysis. The secondary effects of soil pH on other plant nutrients’ availability are probably more important than soil pH per se. For example, P becomes less available to plants at low soil pH (Nobel, 1988). Lime and P are relatively immobile in the soil, and as a result, these need to be thoroughly mixed with the soil before planting. In addition to decreasing vegetative growth and DM (Berry and Nobel, 1985), high soil salinity also decreases water content of the cladode, uptake of K and calcium (Ca), and root-shoot ratio (Nerd et al., 1991b). The addition of gypsum helps to neutralize excess salts in the soil solution. The cactus plant reacts very well to organic manures, improving the soil structure, nutrient content, and water-holding capacity (Inglese, 1995; Singh, 2003). As a general guideline, 6–10 t ha<sup>-1</sup> of well-composted animal manure needs to be incorporated into the soil before planting (Potgieter and D’Aquino, 2017).

### 4.3 Windbreaks

It may be beneficial to plant live windbreaks in windy areas to minimize wind's adverse effects in cactus orchards. Heavy rainfall accompanied by strong wind can cause young plants to lodge and even cause branches and cladodes to break off (Felker et al., 2005). Pollination and plant protection sprays are all negatively affected by high winds; it is difficult to perform orchard practices (pruning, fruit thinning, harvesting) under windy conditions, as the glochids tend to become airborne (Wessels, 1989). When developing cladodes and fruit are too close together on a cladode, chafing during windy spells can damage the fruit. Live windbreaks must be adapted to the area of planting, and they should receive water and plant nutrition to ensure that they do not compete with the orchard. A famous tree for live windbreaks in orchards is the Australian beefwood, *Casuarina* spp. (Potgieter and D'Aquino, 2017).

### 4.4 Planting Time

Roots and cladodes reach their highest growth rate during late spring to early summer (Wessels, 1988b). In summer rainfall areas, newly planted cladodes benefit from rains that occur after planting (Pimienta-Barrios, 1990; Singh, 2006). Planting can be extended to mid-summer in areas with mild winter. The idea is for the plant to become well established and survive cool winter conditions. However, autumn planting is recommended in Morocco (Nasr and Jamjoum, 2002; Nasr, 2015) and Jordan (Homrani Bakali, 2013), where it resulted in a well-established root system in winter, strong growth in summer, and earlier fruiting.

For forage production under tropical conditions, planting usually takes place in the last third of the dry season to reduce the occurrence of plant pathogens, which are more common during the rainy season (Inglese, 1995; Farias et al., 2005). Cactus cladodes planted during the dry season can develop the initial roots after planting, thus reducing disease incidence in the wet season (Mondragón-Jacobo and Pimienta-Barrios, 1995).

Same planting times are also followed for the traditional nopalitos production, unless otherwise plants are grown under protective conditions, such as mini-plastic tunnels, low-tech greenhouses, macro tunnels, or even hydroponic systems. *Opuntia* can be planted at any time of the year (Mondragón-Jacobo and de Gallegos, 2017).

## 4.5 Orchard Planning

### 4.5.1 Row Orientation

Unlike plant density, orientation cannot be changed and fixed for the orchard's lifespan (Potgieter and D'Aquino, 2017). Cactus pear cladodes are inclined to face the east-west direction, except at lower latitudes ( $>27^\circ$ ) and in areas where vegetative growth occurs during winter. In these situations, the row orientation is less important (Nobel, 1982). Once row orientation is decided, it is important to achieve optimal light utilization over the whole tree canopy during the day (García de Cortázar and Nobel, 1991). While the generally preferred row orientation is north-south to capture equal daily solar irradiance under sunlit and cloudy weather conditions, it can be adapted to suit the latitude, site, and incidence of sunburn. There are other practical considerations, such as sloping fields, where it is recommended to plant on the contour to prevent soil erosion (Stassen et al., 1995).

### 4.5.2 Planting Space and Depth

Plantations are established at different density levels according to the type of function and utilization according to the local ecological conditions and to the conditions of management and exploitation (i.e., fruit, vegetable, fodder). In general, single, mature, large, terminal cladodes of uniform appearance, visually free of any defects, insects, and diseases, should be selected as plant material for traditional fruit production operations (Potgieter, 2007). Inglese (1995) recommends placing two parallel cuttings spaced 0.4 m apart at a single planting station for rapid canopy development, or alternatively 3–4 single cuttings positioned in a triangle or square and spaced 0.3 m apart. Although this method has the advantage of faster canopy development, it results in wider within-row spacing and requires large quantities of planting material (Mondragón-Jacobo and Pimienta-Barrios, 1995). Plants are established quite densely, 2–6 m apart between rows and 1–2 m along the row, with about 5000 individual plants per hectare or more (Hedgerow system) (Unterpertinger, 2006). However, in modern plantations, the density is much less: 500–1000 plants per hectare (free-standing trees system) (Potgieter and D'Aquino, 2017). In sandy soils, cladodes need to be planted deeper than in heavier soils to prevent lodging. Likewise, small cladodes should be planted deeper to ensure adequate rooting. There are three ways to plant cactus pear: upright, on the side at a  $30\text{--}45^\circ$  angle, or flat. The upright position is the most commonly adopted (Inglese, 1995) and is preferred for fruit production. Cladodes are planted upright (vertically), with the cut end pointing downwards into the soil. As a result, cladodes will root quickly, forming sturdy developed plants (Arba, 2009; Arba and Benrachid, 2013). Cladodes should be positioned with flat sides towards the working row. Soil needs to be firmed around the cladode after planting to ensure proper contact between the cladode and

the soil. The only disadvantage of this planting method is the possible rotting at the cut end (Wessels, 1988b).

Fodder planting density is governed by the conditions under which the exploitation, harvesting, and transportation are done. A density of 3000–5000 plants per hectare is carried out in traditional cultivation under a direct grazing system or under manual harvesting and draught/burden animal transportation in the case of a cut-and-carry system. Modern plantations, with mechanical cultivation, harvesting, and transportation to the site of consumption by tractors and trailers, are planted at a density of 1000–2000 plants per hectare, with a spacing of 5–7 m between rows and 1–2 m along the rows. Plants could achieve higher biomass productivity at small plant spacing. They can produce up to 47 t ha<sup>-1</sup> year<sup>-1</sup> as irrigated high-density plantation in an open field higher than C<sub>3</sub> and some C<sub>4</sub> plants (Nobel, 1998). Double pads are set in plough furrows. The bottom pad is buried in the furrow and covered with soil materials, drawing a second furrow. They may alternately be set in trenches, which have first been fertilized with farm manure and/or chemical fertilizers (usually superphosphate and ammonium nitrate or urea) (García de Cortázar and Nobel, 1990). If mechanization is available, this should be taken into account when choosing the optimal spacing, which may also vary with location (Dubeux et al., 2011a). In dryer areas, spacing should be greater so that plants can build greater root mass, reducing the risk of water availability during drought periods (Dubeux et al., 2011b). Sites with better soil fertility and greater rainfall are more suited for hosting high-density cactus plantations (Dubeux et al., 2017). Plant spacing in alley cropping should be increased to 10–15 m between cactus plants with perennial crops grown in the interspace. Alley cropping is a variation of hedgerow intercropping. Leguminous and fast-growing tree or shrub species are preferred (Saraiva, 2014).

The traditional open-field system is the most common for food (nopalitos) production. The system relies on bushy plants (<1.80 m), started from single cladodes planted in rows 0.8–1.5 m apart. The individual plants are planted at 0.5–0.75 m apart, resulting in planting densities of 10,000–40,000 plant ha<sup>-1</sup>. The basal or mother cladodes are trained to two or three branches for complete row filling, with partial filling between rows to allow movement and transit between rows. A mini-plastic tunnel system is also used in some small family orchard in Mexico to protect plants from light frost (–1 °C). Tunnels are established on a wide planting bed (1.2–1.5 m), with 3–4 rows of cladodes at a row spacing of 0.3–0.4 and 0.2–0.3 m within the row. The number of rows varies according to the width of the tunnel. A 2-m long fraction of a bed can have 18–24 cladodes. The final planting density varies according to bed width and corridor separation, which depends on the availability of labor and transport. Plastic tunnels are built with arched steel rods (3/8 caliber [0.95 cm]) fixed to the ground, covered by transparent polyethylene sheet (caliber 1.5 cm), and reinforced with polyethylene rope placed diagonally over the tunnel to secure the plastic against the wind. The arches are usually <2 m, designed to cover a single bed of nopalitos. Ventilation is passive, provided by the manual lifting of the plastic cover during the hottest hours of the day. Some other types of protected systems are used in nopalitos production, such as low-tech greenhouses and

macro-tunnels, which are used to protect the plants from high temperature, light frost, and sun radiation (Mondragón-Jacobo and de Gallegos, 2017).

Hydroponics cultivation for fodder or nopalitos production can be adapted to arid areas where water availability for irrigation is restricted with intense pressure on grasslands. Small-scale hydroponic modules could allow the efficient utilization of limited volumes of water and improve nutrient use efficiency and plant production, improving rural income. There are shallow artesian wells and intermittent water sources in many of these areas that can provide enough water to irrigate plant species such as *Opuntia*, characterized by its high water use efficiency and productivity (Nobel, 1998). The system is based on a standard passive cooling plastic-covered greenhouse; it uses sub-irrigation and includes a ground cover on alleys to ensure maximum water saving, optimum usage of fertilizers, and convenient weed control. Plants are established at high density and are kept bushy to reduce crowding and improve light interception through the canopy (Mondragón-Jacobo and de Gallegos, 2017).

#### 4.6 Care of Newly Planted Cladodes

One or two light irrigations (10 L plant<sup>-1</sup>) in a small earthen dam around the plant promote should be enough for root development, but care should be taken to avoid over-irrigation of young plants (Potgieter, 2001). Newly established cladodes may develop fruit soon after planting. Due to the high sink demands of the fruit, it is recommended to remove them in the first year of the plantation (Wessels, 1988b; Inglese, 1995), and a light fruit crop may be left to mature starting from the second year (Potgieter and D'Aquino, 2017).

### 5 Cultural Practices

As an arid-zone plant, *Opuntia* field practices are usually less than those of other fruit trees; however, to get the maximum plant yield, some practices should be applied.

#### 5.1 Fertilization

Fertilization induces a higher yield of fruit and cladodes. Deficiencies in mineral nutrients affect cactus pear plant metabolism, resulting in a negative impact on fruit yield and quality (Nerd and Mizrahi, 1992; Zegbe Dominguez et al., 2014). To make fertilizer recommendations for cactus pear, it is essential to consider the plant nutrient status of the terminal cladodes and the available nutrient reserves in the soil.

Chemical fertilizers are a quick source of nutrients, while manure represents a longer-term and steady nutrient supply. Combining manure with mineral fertilizers gave the best results in fruit orchards. Nutrient elements influence vegetative and reproductive phenology, fruit yield, and cactus pear quality, with macronutrients having the greatest effect on fruit production (Zegbe Dominguez et al., 2014; Arba et al., 2015). Of all the plant nutrients, N is the most limiting nutrient in cacti (Nobel, 1983), with the highest N values found in young fertile cladodes (Nobel, 1988). However, very high N concentrations (>2.2%) in 2- and 3-year old cladodes may result in excessive vegetative growth with accompanying higher input cost, reduced cladode fertility, poor fruit color development, and uneven ripening (Potgieter and Mkhari, 2000; Inglese et al., 2002a). It has been reported that the normal range of P and K in plant tissue should be around 0.06–0.3 and 0.06–3.5%, respectively, for better fruit production (Nobel, 1983, 1988; Arba et al., 2015). Both Ca and K are the most plentiful mineral elements in the cladodes, potentially more abundant than N (Galizzi et al., 2004). Furthermore, Mg in young cladodes can reach levels of 1.47%. Therefore, N, P, K, Ca, and Mg are potentially limiting factors in cactus pear fruit production if cultivated in nutrient-deficient soils (Magallanes Quintanar et al., 2006). Nutrient concentration in cladodes is affected by fruit crop load, cladode position, plant age, plant tissue, and season (Nerd and Nobel, 1995; Gugliuzza et al., 2002a). Additional flower flush can be induced with the application of N just after the removal of the summer harvest. According to Nerd et al. (1993b), the number of flower buds increased with increasing N levels up to 120 kg ha<sup>-1</sup>, while the N concentration in the cladode tissue is positively correlated to the number of flowers formed. Nerd and Mizrahi (1995) found that the autumn flower flush is higher in younger (<6 years) than in older plants. However, high production systems with two fruit harvests in 1 year from the same plant may have additional nutritional requirements (Groenewald, 1996). Fertilizer application should occur when adequate rainfall or irrigation is available (Nerd et al., 1989; Mondragón-Jacobo, 1999). In the Mediterranean climate, fertilization occurs in winter (Barbera et al., 1992a; Inglese, 1995), with fertigation applied throughout the year (Nerd et al., 1991a). Nerd et al. (1989, 1991a) and Ochoa and Uhart (2006) reported that the application of NPK fertilizer in winter increased floral buds' production in the following spring. However, according to Garcia de Cortazar and Nobel (1991), the best time to apply fertilizers was during the warmer months due to higher PPFD in summer. In Mexico, half of N and all P and K fertilizers were applied with irrigation at the onset of floral budburst, but the other half of N was applied after harvest (Zegbe Dominguez et al., 2014). Under rain-fed conditions in summer, half of N and K, and all P fertilizers could be applied directly after fruit harvest, but the remainder towards the end of March (Wessels, 1988b). Calcium was applied at any time during the year, preferably at least after 1 month of N fertilization (Claassens and Wessels, 1997). Increased rates of NPK fertilization along with irrigation to improve yield may lead to relatively high yield (14 t ha<sup>-1</sup>) (Tudisca et al., 2015) with a possibility of alternate bearing incidence (Brutsch, 1979; Pimienta-Barrios, 1990).

Both organic and inorganic fertilizers are generally beneficial in forage production. The reactivation of buds and the increase in the cladodes' size are the

immediate effects of fertilization, which can be advantageously manipulated for forage production (Mondragón-Jacobo, 1994). Cacti often respond better to organic fertilization than mineral fertilization (Mendez Gallegos and Martinez Hernandez, 1988; dos Santos et al., 1996). There is usually interaction between fertilization, plant spacing, and environmental conditions; the larger the plant population, the greater the necessary to fertilization. Greater responses were observed with the combination of higher organic fertilization and higher plant population. Intensive systems may reach annual productivity of  $>50 \text{ t ha}^{-1}$  of DM (Silva, 2012). A combination of organic and mineral fertilizers might be the best option when less manure is available. High N application ( $160 \text{ kg ha}^{-1}$ ) increased the number of new cladodes of *O. engelmannii*. Individual cladodes were slightly thicker, leading to 12% dry weight enhancement per cladode (Nobel et al., 1987). As the main source of organic fertilization, cattle manure should be applied to the soil about 3–4 months before planting (Carneiro and Viana, 1992). At the time of planting, manure can be supplemented with mineral fertilizers for better plant growth. A minimum of  $20 \text{ t ha}^{-1}$  of cow manure every other year, supplemented with annual fertilization of 40 and 90 kg of N and  $\text{P}_2\text{O}_5$  fertilizers, are respectively suggested during the rainy season for best results. These rates can be adjusted according to the fertilizer type and source of N and P. Half of the N amount can be applied early in the season and the rest after 45 days, but P fertilizer can be added once with the first half of the N fertilizer. Both fertilizers can be distributed along the rows and lightly covered with soil (de Souza, 1963; Mondragón-Jacobo and Pimienta-Barrios, 1990). *Opuntia* respond very well to N and P fertilizers showing a yield increase of about 200–300% following moderate fertilization rates. Plants also showed cladode yield increase with organic fertilization even at a very low precipitation rate (150–200 mm) (Monjauze and Le Houérou, 1965; De Kock, 1980; Le Houérou, 1999). Metral (1965) reported that *Opuntia* respond well to N and P but not to K fertilization. Lima et al. (1974) reported that plants responded well with the incremental rates of N and P, up to 100 and 50  $\text{kg ha}^{-1}$  N and  $\text{P}_2\text{O}_5$ , respectively. dos Santos et al. (1996) recorded a 30% increase in *Opuntia* production with the application of 50–50–50  $\text{kg ha}^{-1} \text{ year}^{-1}$  of N- $\text{P}_2\text{O}_5$ - $\text{K}_2\text{O}$ , respectively. González (1989) found that crude protein of forage cactus, *Opuntia lindheimeri*, increased from 4.5% for the control treatment to 10.5% with the application of 224 kg N and 112 kg P per hectare. Nobel et al. (1987) observed that boron (B) significantly increased cactus yield. Nobel (1995) indicated that N, P, K, B, and Na are the nutrients that influence cactus productivity. Baca Castillo (1988) ranked P, N, K, Ca, B, magnesium (Mg), iron (Fe), and manganese (Mn) in decreasing order of importance, considering them to be the nutrients with the greatest effect on cactus growth.

Nopalitos is generally considered a rustic crop, able to survive in poor soils and dry areas. However, if the plant is exposed to good soil, abundant fertilization, and irrigation, productivity is significantly boosted (Mondragón-Jacobo and Pimienta-Barrios, 1990). Zuniga Tarango et al. (2009) stated that  $100 \text{ t ha}^{-1}$  of fresh manure is sufficient to obtain a profitable yield of nopalitos. Aguilar (2007) reported that a total yield of 45–60  $\text{t ha}^{-1} \text{ year}^{-1}$  was obtained with 3.3  $\text{t ha}^{-1}$  of vermin-composted dairy manure, instead of 800  $\text{t ha}^{-1}$  of fresh manure. Blanco Macias et al. (2006)

reported positive interactions between Mg-Ca, Mg-K, and K-P. For a planting density of 10,000 plant ha<sup>-1</sup>, plants need 24 kg N, 71 kg P, 1124 kg K, 954 kg Ca, and 417 kg Mg per hectare for a yield of 564 t ha<sup>-1</sup> of fresh matter or 28 t ha<sup>-1</sup> of DM. Valdez Cepeda et al. (2009) warned of the high response of nopalitos plants to N fertilization and the possibility of nitrate accumulation at toxic levels in cladodes, potentially dangerous if used as forage or vegetable.

## 5.2 Irrigation

The exceptional drought tolerance and high WUE of cactus pear plants (Han and Felker, 1997) are the primary reasons for its popularity as a rain-fed crop in many areas of the world with low rainfall and shortage irrigation water (Zegbe Dominguez et al. 2015). Although the plant can survive in areas receiving 200 mm year<sup>-1</sup> (Acevedo et al., 1983), the optimal rainfall range for cactus pear production is 400–600 mm year<sup>-1</sup>, but soil type also plays a role in the actual plant water requirement (Le Houerou, 1992, 1994). Although large fruit yield can be achieved under low rain-fed conditions (Potgieter, 2007), supplementary irrigation of cactus pear is advisable in summer rainfall areas where <300 mm year<sup>-1</sup> is received (Mulas and D'Hallewin, 1997; Van der Merwe et al., 1997). Irrigation during periods of unfavorable climate, such as dry spells during the rainy season or when spring rains are late, is advantageous (Wessels, 1988d). In a Mediterranean climate, where most rainfall is in winter, supplementary irrigation in summer is required for high yield and good fruit quality (Mulas and D'Hallewin, 1997; Homrani Bakali, 2013). Irrigation of cactus pear is common in Italy, Egypt, Jordan, Morocco, Chile, and the winter rainfall areas of South Africa. There are definite advantages with supplementary irrigation of cactus pear, especially during certain critical phases of plant growth and development (Potgieter and D'Aquino, 2017). Garcia de Cortazar and Nobel (1992), Mulas and D'Hallewin (1997), and Liguori et al. (2013) reported the beneficial effects of irrigation on vegetative plant growth, cladode number, and canopy size. Fruit yield per plant is generally higher in irrigated than non-irrigated plantations, and researchers ascribe higher yield to a higher average number of fruit per cladode than to increase in fruit size (Mulas and D'Hallewin, 1997; Mondragon-Jacobo et al., 1995). Delay in irrigation during winter when annual rainfall is <300 mm resulted in a substantial reduction of cladode fertility and off-season winter crop water shortage, particularly during fruit development period (FDP), which may adversely affect fruit quality (Nerd et al., 1989). Two to three irrigation times of 30–50 mm each during FDP increased fruit size and fruit pulp percentage of cactus pear (Barbera, 1984, 1994; Zegbe Dominguez et al., 2015). However, irrigation alone cannot compensate for a reduced fruit size when there is a high fruit per cladode, making fruit thinning essential to achieve good fruit size (La Mantia et al., 1998; Gugliuzza et al., 2002a). As little as 10 mm rainfall is adequate to wet the soil in the root zone of cactus pear, resulting in the plant being able to efficiently utilize small quantities of rainfall without producing fruit (Nobel, 1995). Due to the plant's



shallow root system, irrigation amounts of 20–25 mm at a time should be adequate (Wessels, 1988b). The crop factor ( $K_c$ ) for cactus pear has been determined within a range of 0.5–0.6 (Consoli et al., 2013). Two to three irrigations (60–100 mm) applied during FDP increased productivity and improved fruit quality (Gugluizza et al., 2002b), while two 50–80 mm applications of water during FDP are essential to achieve export fruit size (Inglese et al., 1995). According to Homrani Bakali (2013), 3 and 6 irrigations per year produced more fruit than just one per year in Morocco. In South Africa, Haulik (1988) suggested  $\leq 3$  supplementary irrigations per year; (1) before budburst is required to stimulate the reproductive flush, (2) then at anthesis to stimulate fruit setting, and (3) in the early stages of fruit development to improve fruit growth. Cactus pear is sensitive to dissolved salts in its root zone, and therefore the quality of irrigation water is important, meaning that NaCl concentration should not be over  $25 \text{ mol m}^{-3}$  to avoid salinity problems (Nerd et al., 1991b).

Irrigation is not a common practice in cactus orchards dedicated to forage production. *Opuntia* use water more efficiently than traditional fodder crops, and their productivity is high compared to the most native vegetation grown under similar conditions (Dubeux et al., 2017). De Kock (1980) reported that plant use about 267 L water to produce 1 kg of dry matter, compared to other fodder crops, such as *Atriplex* sp. that uses about 304 L water to produce 1 kg of DM. Nobel (1988) and Le Houérou (1991a, b) reported that *Opuntias* produce up to 10, 20, and 30 t of DM  $\text{ha}^{-1} \text{ year}^{-1}$  in arid, semi-arid, and sub-humid areas, respectively. Such high yields demand careful crop management and good deep soils. Le Houérou (1994) stated that cactus and other drought-tolerant and water-efficient fodder shrubs could survive under a low amount of rainfall (50 mm) in a particular year, but with neither growth nor production. Therefore, irrigation is required for optimal plant growth and productivity. In some regions where low rainfall associated with warm night temperatures limits cactus development, applying a small amount of water has expanded the cactus planted area. Dubeux et al. (2015) reported that drip irrigation (only 10 mm  $\text{month}^{-1}$ ) resulted in an annual DM yield of  $\leq 19.6 \text{ t ha}^{-1}$  in regions where cactus do not grow well because of the low rainfall and warm night temperature. Salt concentration in irrigation water is a problem. The application of the small amount of water along with organic fertilization reduced any potential salinization problem. In rain-fed systems, rainfall use efficiency (RUE) varies according to the environmental conditions and management practices (Dubeux et al., 2006).

Drip irrigation is an efficient method in Nopalitos orchards (Vasquez Alvarado et al. 2009). The recommended irrigation schedule is four irrigation times when the tensiometer reaches 35 cb, and just two times when it reaches 70 cb. Water requirements also vary according to the location; It was estimated to be  $3.27 \text{ mm day}^{-1}$  for California, but  $1.65 \text{ mm day}^{-1}$  in Mexico (Nobel, 1998). To improve irrigation efficiency, it is necessary to consider the rainfall distribution and avoid excess irrigation rates, using a single dripping line for two consecutive rows (Mondragón-Jacobo and de Gallegos, 2017).

### 5.3 Pruning and Training Systems

The selection of an appropriate pruning and training system for cactus pear is closely related to the planting system, layout, and spacing chosen at the planning phase. In frost-free locations, pruning can be performed at any season. The main reason for pruning in cactus pear is to ensure maximum PAR interception by terminal cladodes. The development of floral buds is mostly observed in mature cladodes that are at least 6 months old (Pimienta-Barrios, 1990). Most terminal cladodes exposed to adequate sunlight will produce flower buds (Nerd and Mizrahi, 1995), while shaded cladodes are usually less fruitful or even infertile (Wessels, 1988a; Inglese et al., 2010). Late pruning, especially in overcrowded plants, will result in cladodes not being exposed to sufficient PAR to make them fertile. Therefore, to ensure high CO<sub>2</sub> uptake and cladode fertility, it is important to prevent excessive cladode shading (Pimienta-Barrios, 1990; Inglese et al., 1994a), particularly during the last 8 weeks before spring floral budburst (Barbera et al., 1993a; Cicala et al., 1997). Other benefits of pruning include; controlled plant size, training of the plant into a hedgerow, increased fruit yield, improved fruit size, easier pest detection and control, easier harvesting and rejuvenation of old plants (Hester and Cacho, 2003; Inglese et al., 2009, 2010). About 20–50% of the terminal cladodes should be removed in pruning (Oelofse et al., 2006). However, excessive pruning will reduce yield and contribute to strong vegetative growth in the following season (Inglese et al., 2002b). All infected, small and damaged cladodes should also be removed (Potgieter and D'Aquino, 2017).

Pruning for training purposes begins in the first year of orchard establishment to direct vegetative growth into the desired plant shape, and this will be switched to productive pruning when the plants start bearing (Targa et al., 2013; Nasr, 2015). In countries where high-density hedgerows are used, plants are pruned to a pyramidal shape (Potgieter, 2001). Where square planting systems with wider spacing are standard, vase or globed-shaped plants are formed (Inglese, 1995). These plants do not have main stems, resulting in large plants with many terminal cladodes distributed around the outer portion of the canopy (Inglese et al., 2002a).

Productive pruning is used to maintain a good balance between vegetative and reproductive growth with an adequate number of new terminal cladodes for the subsequent year's blooming (Mulas and D'Hallewin, 1992). Environmental conditions, cultivar growth habits, and plant spacing affect canopy density (Inglese et al., 2002a). Reduction of the canopy density through pruning facilitated fruit thinning and harvesting and improved fruit quality (Inglese et al., 2010). Plant height should not be over 1.8 m to avoid the use of ladders to perform orchard practices (Potgieter, 2001; Nasr, 2015).

Rejuvenation pruning could be conducted in old cactus pear plantations (25–30 years), senescence of the canopy, and yield reduction with noticeable alternate bearing (Mulas and D'Hallewin, 1992). Rejuvenation of old plants can be achieved by cutting the plant back to a height of 0.5 m above soil level. Only 3–4 well-spaced main scaffold branches should be left for the development of the new

plant. To prevent sunburn, the whole plant should be painted with white polyvinyl alcohol (50%). With a well-established root system, the plant resumes fruiting within 2–3 years after rejuvenation pruning (Wessels, 1988b; Mulas and D’Hallewin, 1992). Newly developed cladodes must be thinned to prevent cladode overcrowding (Potgieter and D’Aquino, 2017).

Annual summer pruning is conducted to reduce the competition between fruit and vegetative growth. However, this practice may result in a heavy crop, which leads to an alternate bearing pattern (Inglese and Barbera, 1993; Inglese et al., 2002b). Summer pruning is not advisable in areas with cold winter because cladodes that developed late in the season would not have sufficient time to harden off before winter and would be subjected to frost damage (Wessels, 1988b). Newly developed cladodes close to flower buds may cause chafing of the fruit epidermis, making it unmarketable due to cosmetic damage (Potgieter, 2001). The best time to prune is after fruit harvest, but not later than 2 months before floral bud break (Wessels, 1988b).

Growing *Opuntia* for forage production needs careful timing of pruning practices. Cladodes stored “on the plant” maintain higher water content than the detached ones, while the need for labor and storage will be reduced. However, it is advisable to remove them just before starting the next growing season to avoid sprouting new buds (Mondragon-Jacobo et al. 2001a, b). Given that fodder production involves partial or total utilization of the vegetative structure, the capacity to produce new cladodes and recover quickly from pruning are the more important features. The cladodes’ size is determined by the genotype (Mondragón-Jacobo, 1999), and to a lesser extent, by the planting layout and soil fertility. For higher biomass yield in cultivated stands, it is preferable to have cultivars with medium-sized cladodes suitable for close planting (Mondragon-Jacobo and Perez-Gonzalez, 2001). During the first 2 years, care involves only the elimination of cladodes growing too close together, which can be used for human consumption (while still tender) or for animal feeding (Lopez-Garcia et al., 2001). Both *Opuntia fuscicaulis* and *Opuntia ficus-indica* cannot produce both fodder and fruit in the same stand in fruit orchards (unless pruning waste is considered). Therefore, *Opuntia* plantations are harvested every 2–3 years as fodder until they produce fruit (De Kock, 2001). Since most commercial cactus fruit orchards receive N fertilization, therefore N content of the pads from these orchards would approach the 9% protein content (Potgieter, 2001). In South Africa, growers obtained 40 t year<sup>-1</sup> pads from the annual pruning of cactus fruit orchards, which could be a significant source of high protein forage for livestock (Felker, 2001).

For nopalitos cladode production, the productivity of the crop should be tightly regulated by pruning practices. Plant training entails modeling it regularly, avoiding branching towards the alley, and maintaining plant height at <1.8 m. Plants are usually allowed to retain 2–3 main branches, configured as “rabbit ears” or “fan” shape, and the remainder is either harvested or discarded. In practice, training is combined with harvesting (Mondragón-Jacobo and de Gallegos, 2017). The plant can be maintained indefinitely in the juvenile stage by continuous pruning, which is the basic crop management tool for *Opuntia* production. If not pruned, the cladodes

will continue growing until autumn, giving rise to flowers at the beginning of spring. The development of floral buds is mostly observed in mature cladodes that are at least 6 months old (Pimienta-Barrios, 1990). A remarkable example of the utilization of pruning waste is the incorporation of freshly sliced cladodes in the field from where they were collected. This practice increases the soil's moisture and organic matter level and suppresses weeds temporarily (Mondragón-Jacobo and de Gallegos, 2017). The application of high manure rates to the soil, associated with pruning, was responsible for the high yield observed in nopalitos production (Nobel, 1994).

#### 5.4 Weed Control

Weed control has been shown to enhance productivity (Felker and Russel, 1988), especially in young plantations (Inglese et al. 1995). In favorable conditions, roots can grow to a depth of 0.3 m and spread horizontally in a radius of 4–8 m (Sudzuki Hills, 1995). Therefore, weeds compete with cactus for nutrients, moisture, and light. The plant sensitivity to weed competition is due to the cactus plant's very shallow root system (Felker and Russel, 1988; Snyman, 2005), where weeds compete at the same soil level for nutrients and water (Dubeux et al., 2017). Nobel and De la Barrera (2003) showed that 95% of mature cactus pear plants develop at a soil depth of 0.4–4.7 m, while Snyman (2006) reported that roots could spread as far as 2.5 m from the stem in 2 years. Weed control is best performed at an early stage of growth, when competition with the cactus pear crop is minimal (Wessels, 1988a; Inglese, 1995). Farias et al. (2005) reported that when weeds were not controlled, cactus production only produced 3 t ha<sup>-1</sup> of DM after 2 years of growth, compared with 12 t ha<sup>-1</sup> using the pre-emergence herbicide application of Tebuthiuron at 2 L ha<sup>-1</sup>. In an in-row hedgerow planting system, mechanical weed control is preferred when cactus pear plants are young due to their sensitivity to herbicides (Potgieter, 2007). However, in square planting systems with free standing globe-trained plants, mechanical weed control is preferred because this training system makes it difficult to work with ordinary farm style implemented between trees (Inglese and Barbera, 1993). In smaller or more traditional farming systems with limited access to herbicides, the soil between the plant rows may be ploughed to clear weeds (Nasr, 2015; Tudisca et al., 2015). In such a case, mechanical weed control is better than no weed control (Felker and Russel, 1988). Due to high labor costs, chemical weed control is the norm in commercial fruit production. A range of herbicides may be used, but farmers are urged only to use products registered in their respective countries, particularly when the fruit is produced for export. Mechanical control is not as efficient as the pre-emergence herbicides; Diuron, Tebuthiuron, or a combination of Simazine and Ametryne. However, if the weeds are potential quality forage material, it is possible to avoid herbicide application instead of making hay to use when feeding the cactus to livestock. This approach, however, resulted in reduced cactus productivity (Farias et al., 2005). Weeds should preferably be controlled to 1–1.5 m on both sides of the planting rows (Potgieter, 2001). Because the early growth of cactus pear is

extremely sensitive to herbicide sprays, spraying should be avoided on windy days. Stems should be shielded during spraying to prevent herbicide damage. Weeds can be mowed and left on the soil surface as mulch to retain moisture and reduce weed growth (Inglese, 1995). Chemical weed control must be avoided in organic fruit production.

## 5.5 Fruit Thinning

Fruit size in cactus pear depends on water availability (Barbera, 1984), cultivar differences (Zegbe Dominguez and Mena Covarrubias, 2010b), fruit development period (Barbera et al., 1992a), mineral nutrition (Ochoa et al., 2002), and cladode fruit load (Inglese et al. 1995). In contrast with many other fruit crops, very few cactus pear flowers abscise, and 95% of the flowers that set become fruit, unless damaged by late winter frost. However, if crop load has not been reduced by fruit thinning, individual fruit size becomes low and whole branches and cladodes may even break off due to the excessive weight. Fruit prices on local and export markets generally depend on fruit size, with larger fruit selling for higher prices. However, heavy fruit thinning (4 fruit per cladode) may substantially reduce total fruit yield by as much as 58% without any fruit size increase, and it could even cause a second re-flowering (Zegbe Dominguez and Mena Covarrubias, 2010a). According to Brutsch (1992), thinned cladodes produced larger fruit than the un-thinned ones, regardless of the number of fruit per cladode. Thus, good fruit size could be achieved with a high fruit-set per cladode, followed by timely fruit thinning to reduce the very high crop load. In addition to high individual fruit mass (Inglese et al. 1995; Nasr, 2015), fruit thinning has other advantages such as; avoidance of branch breaking under heavy crop load (Wessels, 1988a), easy harvesting (Wessels, 1989), regular and early ripening (Inglese et al., 2002b), reduction of alternate bearing (Hester and Cacho, 2003), and improved total soluble solids (TSS) and fruit quality in general (Zegbe Dominguez and Mena Covarrubias, 2010a, b).

Fruit thinning can occur as soon as the spherical fruit buds are distinguishable from the elongated vegetative buds (Wessels, 1988a), but no later than 3 weeks after anthesis, as later thinning does not improve fruit size (Gugliuzza et al., 2002a). Research findings showed that export-sized fruit (>120 g) could only be produced if no more than 6 fruit per cladode is retained (Inglese et al., 1994b). Since cladodes are not equal in size, fruit thinning in South Africa is carried out at approximately 5–7 cm between fruitlets, rather than to a specific number per cladode (Potgieter, 2001). Leaving adequate space between developing fruit ensures less damage to adjacent fruit during harvest, mostly where specialized harvesting secateurs are used. Fruit that develops on the flat sides of the cladode needs to be removed as they tend to have a long fruit stalk, making packing more difficult. Excess fruit can be removed by hand using polyvinyl chloride (PVC) glove and a sharp knife or pruning secateurs (Potgieter and D'Aquino, 2017).

## 5.6 *Out-of-Season Cropping*

Floral induction in most perennial fruit trees is largely synchronized, resulting in a single harvest at a specific time of the year (Liguori and Inglese, 2015). However, one of the most remarkable characteristics of cactus pear is the plant's capability to re-flower at different times in the same season (Inglese, 1995; Inglese et al., 2002a), either naturally or after inductive practices have been applied (Nerd and Mizrahi, 1997). Out-of-season fruit is sold at substantially higher prices than those of the normal summer season (Mondragon-Jacobo et al., 2009). Successful application of crop manipulation techniques, such as 'scozzolatura' and 'winter production', has considerably increased the provision of fruit from 5 to 9 months of the year on the local fresh produce markets of South Africa, although the volume is limited from May to September (Inglese, 1995; Liguori and Inglese, 2015).

### 5.6.1 *Scozzolatura*

This technique has been accidentally discovered by Coppoler in 1827 (Barbera et al., 1992a). It is a standard crop practice in Italy's cactus pear fruit industry (Barbera, 1995). Complete removal of all newly developing flowers and cladodes of the spring flush resulted in a second re-flowering, approximately 12–16 days later, with fruit ripening 6–8 weeks after the spring flush (SF) (Brutsch and Scott, 1991; Barbera et al., 1992b). Although the second flush usually sets fewer flowers than the SF, the fruit is marketed when prices are higher, compensating for the lower fruit yield (Boujghagh and Bouharroud, 2015). The advantages of scozzolatura include; more complex and compact plant architecture, in addition to more fertile terminal cladodes, and higher fruit yield when practiced from an early plant age (Potgieter and D'Aquino, 2017); increased prices and improved fruit quality, such as fruit size, lower seed-to-pulp ratio, higher pulp percentage (Boujghagh and Bouharroud, 2015; Hammami et al., 2015), greater flesh firmness, more intense pulp coloration (Mondragon-Jacobo et al., 1995).

The re-flowering index (RFI) (i.e., the ratio of the second flush to first flush flowers) may vary greatly depending on the timing of SF removal and the environmental conditions at removal time (Inglese, 1995). Indeed, scozzolatura can also have disadvantages, including; reduced yield, higher peel percentage (Mondragon-Jacobo et al., 1995), possibly due to reduced temperatures during FDP (Inglese, 1995; Hammami et al., 2015), increased peel cracking, poorly colored fruit, lower TSS and lower titratable acidity (Inglese, 1995; Mulas, 1997). The number of cladodes produced with scozzolatura maybe 10–40% less than that of the SF and fruit yield can be as much as 50% lower than in the summer season (Nerd et al., 1991a; Inglese, 1995).

A maximum of 25% of the spring season cladodes should be kept on the plant after scozzolatura, as a higher percentage reduces the RFI of the following spring and promotes biennial bearing (Inglese et al., 2002b). Climatic conditions, cultivar

response, and time of flush removal are essential factors affecting scozzolatura. Environmental conditions at removal time influence the RFI and may cause considerable annual variation in the re-flowering response (Barbera et al., 1991; Nieddu and Spano, 1992). For example, a lower RFI may be obtained if high temperatures coincide with bud initiation, which will result in more vegetative than reproductive buds (Nerd et al., 1989; Nobel and Castaneda, 1998). In some countries, scozzolatura is performed with irrigation (Inglese et al., 2002a), N fertilization (Flores Valdez, 2003), or with fertigation applied at the time of SF removal (Nerd et al., 1993b). It is essential to select the most suitable cultivar for the technique, as re-flowering may be low or even absent in some cultivars (Mondragon-Jacobo et al., 2001a; Targa et al., 2013). The time of SF removal affects the RFI, the ripening time, and fruit characteristics (Barbera et al., 1992b). Inglese (1995) reported a difference in the RFI of between 0.7 (for pre-anthesis flower removal) and 0.5–0.3 (for post-anthesis flower removal). Pre-bloom removal produces the highest RFI (Brutsch and Scott, 1991), but the latest removal stage normally gives the highest economic return, although fruit yield may be lower than that for other SF removal times (Boujghagh and Bouharroud, 2015). Scozzolatura is also regularly practiced in South Africa (Brutsch and Scott, 1991), Morocco (Boujghagh and Bouharroud, 2015), and Tunisia (Aounallah et al., 2005; Hammami et al., 2015). In other parts of the world, scozzolatura produced poor results. For example, scozzolatura under Mexican conditions with ‘Cristalina’ and ‘Reyna’ cultivars gave negative results (Mondragon-Jacobo et al., 1995). Ochoa et al. (2009) reported a very low RFI of 0.05 in Argentina with ‘Amarilla sin espinas’ cultivar. Following the scozzolatura performed at SF removal, the process can be repeated with the complete removal of the first scozzolatura cladodes and fruit (Inglese et al., 2010). Liguori et al. (2006) stated that the double removal of new fruit and cladodes induced the third flush of flowers and cladodes in late August, with fruit production ripening in winter (January–March) in the Northern Hemisphere. Winter fruit obtained by double scozzolatura and covered under PVC polymeric film in late autumn were regular in size and flesh percentage but with slightly lower TSS. However, the RFI was low (20–40%) (Liguori and Inglese, 2015). The low temperature in December stop fruit growth and ripening; therefore, for normal fruit growth and development, it is necessary to cover the plants with PVC tunnels (Liguori et al., 2006).

## 5.6.2 Winter Fruit Production

The flowering of the cactus pear plant is not restricted to spring. A smaller budburst occurs naturally in California (Curtis, 1977), Chile (Sudzuki Hills et al., 1993), and Argentina (Inglese, 1995), as well as in the hot subtropical areas of South Africa (Groenewald, 1996; Potgieter, 2001). In addition to the natural out-of-season budburst in areas with mild winter, a second flowering flush can be obtained. Nerd et al. (1993b) and Nerd and Mizrahi (1994) showed that following the main summer crop harvest, immediate irrigation, and N application at a rate of 120 kg ha<sup>-1</sup> produced an autumn budburst. The production of flower buds increased with increased N

application rates and was positively correlated with the soluble reduced N content in the terminal cladodes (Nerd and Mizrahi, 1994). Although the winter crop gave a 50–80% smaller yield than the main summer crop (Nerd et al., 1993b), higher prices were obtained (Mondragon-Jacobo and Perez-Gonzalez, 1996). Groenewald (1996) reported that even without irrigation, this technique could be successfully applied under rain-fed conditions such as in summer rainfall areas of South Africa.

Furthermore, the flowering response to N is affected by plant age. Floral bud production is much higher in young plants ( $\leq 6$  years) than in older plants (Nerd and Mizrahi, 1994). However, this technique is only feasible where winter temperatures are sufficiently high for fruit development (Nerd et al., 1993b). The peel-to-pulp ratio is higher in winter than in summer fruit due to the thicker peel. Cultivars producing high fruit yield in summer (e.g. ‘American Giant’) do not respond to the applied N. Besides, pruning should be delayed until after winter fruit ripening, despite by this time flower buds of the main summer crop has already appeared, which making pruning difficult (Groenewald, 1996).

### 5.7 Disease Control and Orchard Sanitation

Pruned cladodes during winter pruning, cladodes that break off during normal orchard practices, and fruitlets removed during thinning should be removed from the orchard regularly and destroyed. Detached cladodes and thinned fruitlets should not be dumped near the orchard, as they form roots and begin to grow, serving as host plants for various diseases, which result in increased plant protection costs (Potgieter, 2001). The damages of most pests will be limited with continuous pruning. The most serious pests in all types of *Opuntia* plantations are; the cochineal insect *Dactylopius opuntiae*, the cactus moth *Cactoblastis cactorum*, and various Lepidopteran insects, such as the zebra worm *Olycella nephelepsa*, the white worm *Megastes Cyclades*, and the cactus pad joint borer *Metapleura potosi*. Similarly, cactus weevil *Metamasius spinolae*, shot hole weevil *Gerstaeckeria spp.* and areole weevil *Cylindrocopturus biradiatus*, and earthworms *Phyllophaga* sp. are also serious pests in nopalito orchards planted in light volcanic soils (Mondragón-Jacobo and de Gallegos, 2017).

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# Chapter 7

## Molecular Characterization of *Opuntia* spp.



**Stefano La Malfa, Giuseppina Las Casas, Marco Caruso, Gaetano Distefano, Alessandra Gentile, and Elisabetta Nicolosi**

**Abstract** According to the different classification methods, the genus *Opuntia* belongs to the *Cactaceae* family native to the Americas, and encompasses several species ranging from 75 to 250. Many of these species are diffused, and some of them are cultivated in arid and semi-arid areas. The most economically important species is *O. ficus-indica* L. (Mill.), but the genus includes other important edible species (from diploid to octoploid), which are spread, wild, or cultivated in many regions worldwide. *Opuntia* species are gaining popularity in different areas, mainly arid and semi-arid, both for fruit production for human consumption and mainly for animal feeding using the tender and mature green part of the whole plant (cladodes). The taxonomic classification of the genus is hampered by several factors: the lack of reliable morphological descriptors, the recurrent intra- and intergeneric hybridization, the frequent polyploidy, and the phenotypic variation displayed by the genotypes under different ecological conditions. Due to the ambiguity of the different taxonomic hypotheses formulated based on morphological traits, in the last years, several DNA-based studies, relying on different categories of molecular markers, have been carried out in order to better assess variability level within the main cultivated species (*O. ficus-indica*) and to assess relationships and origin of the main species.

The first set of studies aimed to characterize the genotypes cultivated for fruit production and the results achieved gave evidence of a narrow germplasm base involved in their origin compared to that of ornamental and wild species. Other studies evidenced a high similarity between *O. ficus-indica* and *O. megacantha*

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suggesting the latter could be a progenitor of the cultivated prickly pear varieties. Cytoplasmic markers were also employed on *Opuntias*; this approach evidenced a multiple maternal phylogeny for the fleshy fruit varieties classified as *O. ficus-indica* and revealed the most common varieties' narrow genetic base used for fruit production. In contrast, greater variability was revealed for genotypes used as forage. Several results converge on the hypothesis of a polyphyletic origin of *O. ficus-indica*; this is likely composed of several originary clones, selected and vegetatively propagated from different parental species for their favorable agronomic traits. However, many *Opuntia* species' different polyploidy levels hampers their clear phylogenetic assignment based on nuclear markers. Extranuclear molecular markers, located in the chloroplast and mitochondrial genomes, have also been employed to investigate the phylogenetic relationship among cultivated genotypes, wild accessions, and related species for the lack of recombination and maternal haploid inheritance and appear to be suitable for overcoming problems related to polyploidy.

The main results from molecular studies will be reviewed in this chapter highlighting their potential for overcoming the faultiness of the taxonomy currently adopted. An integrated approach of molecular-based characterization and the adoption of accurate large-scale phenotyping of existing germplasm collections could lead to functional management of genetic resources for their sustainable conservation and plan future breeding strategies for improved genotypes to be cultivated in distinct areas for different purposes.

**Keywords** Cactus pear · Molecular markers · Characterization · Breeding · Germplasm collection

## 1 Introduction

*Opuntia* genus belongs to the *Cactaceae* and includes a vast number of species that originated from the Americas. According to the different classification criteria, the number of species ranges from 75 (Hunt et al., 2006) to 160 (Gibson & Nobel, 1986) or 250 (Britton & Rose, 1963). Several *Opuntia* species have been cultivated for centuries in many countries, but they are also considered among the most highly invasive species in some arid and semi-arid areas, especially in the Mediterranean region, in Australia, and Africa (Freeman, 1992; Vila et al., 2003).

Cultivated opuntias include *O. ficus-indica*, *O. megacantha*, *O. streptacantha*, *O. albicarpa*, *O. amyclaea*, *O. robusta*, *O. hyptiacantha*, and *O. cochenillifera* (syn. *Nopalea cochenillifera*, grown in Mexico as a forage crop) among others (Pimienta-Barrios, 1994; Scheinvar, 1995; Kiesling, 1998; Mondragòn-Jacobo, 2001). The most popular and economically important species is *O. ficus-indica* L. (Mill.) (Fig. 7.1), mentioned with various common names as cactus pear, prickly pear, Indian fig, Barbary fig, etc. This species domesticated about 9000 years ago, in the central region of Mexico and then diffused in warm regions all around the world by



**Fig. 7.1** Particular of plant, cladodes, flowers and fruits of *Opuntia ficus-indica*

European travelers, starting from the late fifteenth century (Kiesling, 1998; Griffith, 2004). In Europe, it was, in the beginning, used as an ornamental plant and a curiosity in botanical gardens and then diffused as a crop matching several human needs.

This species' popularity is due to its fruits and its stems, used for animal feeding or human consumption as a fresh vegetable (Casas & Barbera, 2002). However, the opuntias have gained popularity also for ornamental purposes and derivate products for cosmetic and medical uses (Mondragòn-Jacobo, 2001). Most of the varieties used for fruit production in native lands, and other productive areas, belong to the species *O. ficus-indica* L. (Mill.) and are known with different names according to specific fruit traits, especially peel flesh color (Mondragòn-Jacobo, 2001).

As intended for human consumption (for salads and pickled products), the cladodes are mainly produced in Mexico, while in other areas of cultivation, the fruit represents the main product. Forage derived from *Opuntia* spp. is an essential food source to feed cattle and sheep, particularly in semi-arid environments and in dry seasons (Mulas & Mulas, 2004; Nefzaoui & El Mourid, 2010; Beccaro et al., 2015). The cladodes can be used either as fresh or stored as silage before their usage (Castral et al., 1977). These species also play a strategic role in ecosystem conservation, showing high adaptability to degraded ecosystems characterized by limited

resources. From the ecological point of view, cactus pear plants were identified as suitable crops for preventing long-term ecosystem degradation (Bariagabre et al., 2016).

The taxonomic classification of the *Opuntia* genus is drastically complicated because of the high level of phenotypic variation under different ecological conditions, the lack of reliable morphological descriptors, the incidence of polyploidy, the occurrence of both vegetative and sexual reproduction, and intra- and intergeneric hybridization (Scheinvar, 1995; Wallace & Gibson, 2002; Caruso et al., 2010; Majure et al., 2012). The nomenclatural problems occurred not only in *Opuntia* but also within other genera of the *Opuntioideae* family.

Opuntias are often divided into *Cylindropuntias* and *Platyopuntias*. *Cylindropuntias* are shrubby species with cylindrical stems, on the contrary, *Platyopuntias* have flattened stems called cladodes (Gibson & Nobel, 1986), and it is the group including agronomically important species cultivated as both fruit and forage crops.

Morphological traits, primarily the presence of spines in the cladodes, were used to differentiate *O. ficus-indica* from other arborescent opuntias such as *O. leucosthrica* (Fig. 7.2) (Nieddu & Chessa, 1997; Kiesling, 1998; Felker et al., 2005; Beccaro et al., 2015). Nevertheless, the taxonomical concept of *O. ficus indica* is somewhat uncertain, and it is sometimes described as a spineless form systematically separate from other cultivated opuntias, such as *O. megacantha*, *O. streptacantha*, and *O. amyclaea* (Britton & Rose, 1963; Scheinvar, 1995; Reyes-Aguero et al., 2005). In other cases, spiny cultivated accessions have been included in *O. ficus-indica* (Gibson & Nobel, 1986; Pimienta-Barrios, 1994; Felker et al., 2005). However, the presence of spines in the cladodes cannot be considered an excellent character to discriminate *O. ficus-indica* from other arborescent opuntias (Nieddu & Chessa, 1997; Kiesling, 1998; Felker et al., 2005), and it has been speculated that the spineless form of *O. ficus-indica* (absent in wild environments) could have been derived after a selection process in cultivation along years.

The characters traditionally taken into consideration for a phenotypic description of the species (the growth habit, the presence of spines, the number of spines per areole, and the number of areoles) exhibit a high degree of variability within the genus and often changed in the different growing regions, so that they cannot be considered reliable indicators (Rebman & Pinkava, 2001).

## 2 Polyploidy in *Opuntia* Species

Chromosome number and ploidy level are useful in plant taxonomy for genus and species identification. *Opuntioideae* polyploidy is a prevalent feature, and within the family, ploidy levels range mainly from diploid to octoploid (Felker et al., 2006), with the polyploid species representing more than 64% of the total (Pinkava et al., 1998). Polyploidy is considered as one of the major causes of speciation in the *Cactaceae* family (Pinkava, 2002) and, although it hampered a clear phylogenetic

**Fig. 7.2** Cladodes of *Opuntia leucothrica*, a cactus pear related species



assignment of genotypes based on nuclear markers, some studies suggested polyphyletic origin of the most important cultivated accessions for fruit production (Griffith, 2004; Caruso et al., 2010; Las Casas et al., 2018). *Opuntia* genus has a basic chromosome number of  $x = 11$  and different levels of polyploidy, varying from  $3x$  to  $30x$ ; among these, tetraploid ( $4x$ ) and hexaploid ( $6x$ ) species are the most frequent (Pinkava et al., 1985; Segura et al., 2007; Majure et al., 2012). A cariological study on 6 *Opuntia* species showed in all accession endopolyploidy, defined by the presence of nuclear populations of 2, 4, and 8C ( $C =$  cycles necessary to reach the corresponding ploidy level divided by the total number of nuclei measured) in cells of the stem parenchyma. The presence of endopolyploidy and polyploidy in prickly pear provides these plants with adaptive advantages in arid and semi-arid environments (Palomino et al., 2016).

Polyploidy is also favored by the frequent hybridization between *Opuntia* species cultivated or in natural conditions (Grant & Grant, 1982; Griffith, 2003). Different levels of ploidy, including octo-, hepta-, hexa-, penta- and diploid, were reported for *O. ficus-indica* that is considered either an allopolyploid originated from 2 species with  $2n = 44$  or an autopolyploid (Carpio, 1952; Griffith, 2004).

Varieties with high chromosome numbers ( $2n = 6x = 66$  and  $2n = 8x = 88$ ) are mostly found among cultivated genotypes, except a wild population of *O. streptacantha*. Several studies evidenced that both the spiny and the spineless forms of

**Table 7.1** Ploidy levels reported for some *Opuntia* species (modified from Caruso et al., 2010)

Species	Ploidy level
<i>O. ficus indica</i> (L.) Mill.	8X <sup>a,b</sup>
<i>O. megacantha</i> Salm-Dyck	6X <sup>c</sup> /8X <sup>a,d</sup>
<i>O. streptacantha</i> Lemaire	2X <sup>c</sup> /8X <sup>a,e</sup>
<i>O. albicarpa</i> Scheinvar	8X <sup>a</sup>
<i>O. joconostle</i> F.A.C. Weber ex Diguët	8X <sup>a</sup>
<i>O. vulgaris</i> Mill.	2X <sup>b</sup> /3X <sup>b</sup> /6X <sup>c</sup>
<i>O. robusta</i> Wendland	2X <sup>f</sup> /4X <sup>a</sup> /8X <sup>a</sup>
<i>O. spinulifera</i> Salm-Dyck	4X <sup>a</sup> /6X <sup>c</sup>
<i>O. oligacantha</i> Forster	6X <sup>a</sup>
<i>O. elizondoana</i> E. Sánchez et Villaseñor	4X <sup>a</sup>
<i>O. leucotricha</i> DC.	4X <sup>b</sup>
<i>O. cochenillifera</i> Mill. ( <i>sin. Nopalea cochenillifera</i> (L.) Salm-Dick)	2X <sup>b</sup>
<i>O. quimilo</i> Schum.	2X <sup>c</sup>
<i>O. amyclaea</i> Tenore	8X <sup>d</sup>
<i>O. subulata</i> (Muehl.) Engelm.	6X <sup>b</sup>
<i>O. polyacantha</i> Haworth	2X <sup>g</sup> /3X <sup>g</sup>

Ploidy level information taken from: <sup>a</sup>Segura et al. (2007), <sup>b</sup>Fedorov (1969), <sup>c</sup>Moore (1977), <sup>d</sup>Goldblatt and Johnson (1990), <sup>e</sup>Goldblatt and Johnson (2006), <sup>f</sup>Goldblatt (1981), <sup>g</sup>Stockwell (1935)

*O. ficus-indica* are octoploid (Felker et al., 2006), while cultivars with low chromosome numbers ( $2n = 2x = 22$  and  $2n = 4x = 44$ ) are found in wild populations (Pinkava et al., 1992). The same pattern of ploidy was observed by Barbera and Inglese (1993) in samples from Italy. However, for the same species, different chromosome numbers have been reported (Pinkava, 2002; Majure et al., 2012). Despite the studies, the species' ancestry is unknown, many authors reported that it was difficult to correctly assign cultivated genotypes to a defined taxon (Kiesling, 1998; Mondragòn-Jacobo, 2001; Labra et al., 2003; Felker et al., 2006). Table 7.1 reports the ploidy levels of some opuntia species.

### 3 Origin, Diffusion and Genetic Resources of *Opuntia ficus-indica*

*O. ficus-indica* represents the most important species in agricultural economies in arid and semi-arid parts of the world. Despite the interest in its cultivation, little is known about its origin and phylogeny. *O. megacantha* and *O. streptacantha* have been considered *O. ficus-indica* ancestors, but these two species are often confused (Leuenberger, 1978).

According to Griffiths (1914), *O. megacantha* (Fig. 7.3) could be a wild thorny form of cultivated *O. ficus-indica*; this hypothesis was then discussed by Griffith (2004), who evidenced some relationships among the two species. Several authors



**Fig. 7.3** Cladodes of *Opuntia megacantha*, the most probable *O. ficus indica* ancestor



(Benson, 1982; Gibson & Nobel, 1986; Brutsch & Zimmermann, 1993) considered *O. megacantha* as a cultivated taxon and a synonym of *O. ficus-indica* in the “spiny form,” and Kiesling (1998) suggested *O. megacantha* as a reversion to spined plants from spineless *O. ficus-indica*.

Most of the above-reported hypothesis move from the observation of the presence and size of spines. This represents a very variable character since it has been observed that spineless forms are likely due to a domestication process. The opposite process (spination or spine formation) could have also occurred especially in sexual propagation, but also as a mutation in response to stresses (Griffiths, 1914; Le Houérou, 1996; Kiesling, 1998), suggesting that the presence of spines cannot be considered a valuable character in *Opuntia* taxonomy, because the formation of spines is positively related with environmental factors (Labra et al., 2003). In other terms, the presence/absence of spines cannot be considered a reliable discriminating character to separate *O. ficus-indica* from other species, being the presence of spines one of the phenotypes of the species.

According to Griffith (2004), *O. ficus-indica* ancestors derive from a wide area in central Mexico. *O. ficus-indica* was then diffused to other warm climates regions worldwide. In some of these areas, this (and to a lesser extent some other related species) underwent a spreading and naturalization process, especially in Mediterranean countries, becoming a peculiar component of the landscape. As a consequence of sexual propagation, this distribution led to the diffusion of a new form of the species, including spiny forms, and some of these were described as new species (Kiesling, 2013). In the eighteenth century, *O. ficus-indica* was introduced to other continents by navigators who used fruits rich in vitamin C to prevent scurvy

(Diguët, 1928). When introduced in areas with suitable climatic conditions, the species underwent a diffusion process both *via* seeds and *via* cladodes till its naturalization, and this process occurred similarly, but independently, in different environments. The areas where this process happened represent secondary centers of differentiation of the species, in which new hybrids and clones arose and further diffused, all of them sharing a common pool of alleles but exhibiting slight morphological and physiological differences among each other.

The higher intraspecific variability for this species is hosted in Mexico. Here, only considering fruit production, more than 50 varieties and clonal selections are cultivated. Among these, about a dozen are those with a relevant diffusion, including the varieties Amarilla, Blanca de San José, Burróna, Copena, Cristalina, Esmeralda, Fafayuco, Liria, Montesa, Naranjona, Reyna, Roja Lisa. Most Mexican varieties have spines on fruit and cladodes, whereas the varieties spread in Italy (Sicily) are spineless but with glochids on the fruit (Fig. 7.4). The main Italian varieties are differentiated based on the fruit color: Bianca (white color), Gialla (yellow color), and Rossa (red color) and some clones, the so-called Trunzare, with crispy pulp. The variety Gialla is the most diffused (about 90% of the production) for its resistance and suitability to be forced for extra-season production after a summer pruning. A similar varietal composition is present in African countries (mainly North and East Africa) and near East. Other important producing countries (Argentina, California, Chile, Israel, and South Africa) have their varieties often identified based on the fruit color or some imported ones. However, no variety can be considered suitable for different areas, nor any important nursery activity, leading to the diffusion of selected varieties such as other fruit tree species.

The *Opuntias* play an essential role as a forage crop in dry regions since the cladodes, with high-water content, help provide animals' water needs. The *Opuntias* cultivation for forage production is increasing globally, reaching 2.6 million hectares. The countries mainly involved are Brazil and Tunisia with 600,000 ha both, followed by South Africa with 525,000 ha (Grünwaldt et al., 2015). Thornless varieties are preferred for forage production for the ease of consumption and digestion by animals. *Opuntias* forage has many advantages, including high palatability, high digestibility, easy management of cultivation, essential food for livestock and wildlife in arid and semi-arid areas, resistance to transport conditions, high rate of adaptability to harvesting conditions, evergreen and high biomass production.

The development of thornless varieties of *Opuntia* for forage began in the early twentieth century in California. Fuentes (1991) and Flores Valdez and Aranda Osorio (1997) indicate the use of about 18 species, 15 of which are *Platyopuntiae*, as the most important in terms of number, distribution, and preference of the farmers, these including *O. streptacantha*, *O. megacantha*, *O. leucothrica*, *O. robusta*, *O. cochellinifera*, *O. lindheimeri*, *O. engelmannii*, *O. cantabrigiensis*, and *O. ficus-indica*. Felker et al. (2006) refer to about 200 spiny and spineless accessions belonging to 17 species, cultivated in Brazil, South Africa, and the USA, although the most used are *O. engelmannii* and *O. lindheimeri* (De la Cruz, 1994).

The first real breeding program for forage varieties started in 1980 at the Agronomic Institute of Pernambuco (IPA) in Brazil. Through the introduction and



**Fig. 7.4** Typical Sicilian cactus pear orchard on the slopes of Mount Etna (a) and fruits in the market (b)

generation of new accessions, the increase in genetic diversification has led to a germplasm bank of about 1400 accessions (Santos et al., 2006). Twenty-eight genotypes from this collection were analyzed by ribosomal RNA ITS markers, separating the accessions in *O. cochenillifera*, *O. robusta*, and *O. ficus-indica* according to the botanic classification (De Lyra et al., 2015). Today in Brazil, the most cultivated varieties are Orelha de elefante Mexicana (*Opuntia stricta* Haw), Orelha de onça,

Palma miuda with its clones Ipa Sertania and Mao de Moça (*O. cochellinifera*), Gigante, Palma Redonda, Copena f1 and Copena v1, all spineless clones of *O. ficus-indica* or *O. robusta*.

Given the difficulties in formulating robust taxonomic hypotheses based on phenotype, significant attention was paid to molecular studies' contribution to clarify different aspects of origin and phylogeny of *Opuntia* species and, in particular, of *O. ficus-indica*.

## 4 The Contribution of DNA-Based Studies

The main limit of phenotypic identification is related to the phenotype's strong environment interaction with an environment that makes misleading classification. Since morphological traits are subject to a continuous variation, linked to the environmental conditions, and different species have relative ease of cross-hybridization (with the presence of individuals with intermediate characteristics for most of the morphological traits), there is a lack of specific descriptors for *Opuntia* species, that has led to an erroneous species designation for many accessions (same varieties classified as different species or considered to be hybrids among unknown parentals).

To overcome these problems, molecular markers, which are reproducible, stable, and unconnected to the environmental context, can be used to clarify taxonomical classification. In the past, studies have been performed to characterize *Opuntia* germplasm using random and specific molecular markers, including RAPDs, nrITS, AFLP, ISSR, SSR, and most of the DNA-based analyses revealed discrepancies between molecular characterization and classification based on morphological traits.

The first obstacle in applying DNA-based analyses in *Opuntia* was related to the optimization of nucleic acid extraction, especially for the absence of leaves. Several protocols were so developed taking into account the possibility to extract DNA from different sources, including roots and cladodes, despite the presence of mucilage, polysaccharides, and polyphenols in these tissues (Mondragòn-Jacobo et al., 2000; Griffith & Porter, 2003). Over time, the availability of several reliable protocols and then commercial kits have made it possible to extract high-quality DNAs to be used for PCR-based analyses.

So far, many molecular markers have been employed in *Opuntia* for genotyping individuals and to investigate the phylogeny and the genetic structure of germplasm collections in different countries and, in some cases, in order to discover synonyms or for parentage determination. However, it must be considered that similar to what happened in several other minor fruit species, the development of molecular markers, especially with specific sequences, has been limited.

In 1998, Wang et al. demonstrated the feasibility of DNA fingerprinting in *Opuntia* using RAPDs. The same molecular markers were then used to analyze germplasm collections in Mexico and many other countries ((Mondragòn-Jacobo, 2003; Zoghiami et al., 2007; Bendhifi et al., 2013). These markers were also used to identify the somatic origin of some Mexican accessions (Mondragòn-Jacobo, 2002)

and for the identification of putative clones within a germplasm bank collection in Mexico (García-Zambrano et al., 2006). The same authors also coupled RAPD markers with amplified fragment length polymorphism (AFLP) markers, and some of the results were not confirmed (García-Zambrano et al., 2009).

Zoghalmi et al. (2007) analyzed with RAPD markers *O. ficus-indica* ecotypes from a Tunisia collection, highlighting the lack of relationships according to the different geographical origin. RAPDs were also employed together with ISSRs markers, and a vast number of different species were characterized (Valadez-Moctezuma et al., 2015). In particular, the species *O. ficus-indica*, *O. albicarpa*, and *O. megacantha* were grouped. Similar results were obtained by Samah et al. (2015), who reported the lack of clear distinction when compared *O. ficus-indica* with *O. albicarpa*, *O. hyptiacantha*, *O. lasiacantha*, *O. megacantha*, and *O. streptacantha*.

AFLP markers have also been used to clarify genetic relationships in Tunisian germplasm collections, revealing a high genetic similarity within *O. ficus-indica* accessions (Snoussi Trifa et al., 2009). The level of genetic diversity was also investigated by ISSR in cactus clones from Brazil, revealing a low genetic differentiation level (Souto Alves et al., 2009).

Two essential studies (Labra et al., 2003; Griffith, 2004) employed different molecular tools to elucidate taxonomical aspects of the genus, particularly the origin of *O. ficus-indica*. Labra et al. (2003) used AFLP to verify the non-existence of genetic differentiation between *O. ficus-indica* and *O. megacantha* populations and suggested that *O. ficus-indica* should be considered a domesticated form of the spiny *O. megacantha*.

On the other hand, Griffith (2004) investigated the origin of *O. ficus-indica* through the use of Bayesian phylogenetic analyses of nrITS DNA sequences and considered the species to be a group of different clones, selected for their low number of spines and their fleshy fruits, that were derived from different parentals, most likely from other arborescent opuntias from central and southern Mexico. Griffith's study's results evidenced the presence of a relatively robust clade that includes *O. ficus-indica*, *O. streptacantha*, *O. tomentosa*, *O. leucotricha*, and *O. hyptiacantha* and supported the hypothesis of both central Mexico as domestication center and polyphyletic origin of *O. ficus-indica*. This origin could be due either to hybridization, to the derivation of clones from various parental stock, or lineage sorting of different internal transcribed spacer (ITS) copies in an ancestral population from which *O. ficus-indica* may be derived (Griffith, 2004).

Analysis of genetic variability was also conducted with ISSRs on Brazilian collection of *O. ficus-indica* mainly used as cattle feed (Souto Alves et al., 2009) and Greek fruit varieties (Ganopoulos et al., 2015). In more recent years, SSRs markers were highly informative and appropriate for the characterization of *Opuntia* accessions, and they were used in many characterization studies. These markers can display a very high variability and have a codominant nature.

The first *Opuntia* specific markers were developed in *O. echios* and were later used for genotyping of other *Opuntia* species. In a study of 2009, Helsen and colleagues applied these markers to discriminate between two morphologically distinct

*O. echios* botanical varieties (*echios* and *gigantea*) native to the Galapagos Islands. They highlighted the relatively high morphological divergence found on these endemic *Opuntia* species associated with low genetic variability as evidence of accumulation of differences between closely related populations within a species, leading to speciation and adaptation to local environments. The same authors speculate that molecular data do not support morphology-based taxonomic differentiation between *Opuntia* accession.

A new set of microsatellite *loci* was isolated in different species and varieties of *Opuntia* and then used to characterize accessions from Italy and Argentina (Erre et al., 2011; Chessa et al., 2013). These analyses carried out with five robust SSR allowed detecting many alleles useful for both inter- and intraspecific classification of *Opuntia* species.

Samah et al. (2016) used SSR markers and DAPC and STRUCTURE analyses to study the genetic diversity of Mexican *Opuntia* germplasm, considering two variants: sweet fruit (tunas) and acid fruit (xoconostles), to explore the genetic relationships among them. Morphologically, a clear separation between the two types of fruits was reported when the differences in levels of sugars, the portion of the pulp, and the distribution and size of seeds were considered. Genetically, the separation between tunas and xoconostles is contradictory. The existence of a separation between the two fruit types is sometimes reported (Valadez-Moctezuma et al., 2015; Samah et al., 2015), but in other studies rejected (Espinoza Sánchez et al., 2014). In this case, all analyses showed that the distribution of genotypes was not following the current taxonomic assignment of accessions, nor according to end-use or level of domestication.

Microsatellite markers revealed a low level of genetic diversity among Portuguese *Opuntia* spp. populations from *O. ficus-indica*, *O. elata*, *O. dillenii*, and *O. robusta* (Reis et al., 2018). These markers allowed to reveal several other ambiguities in the taxonomic classification of *Opuntia* species.

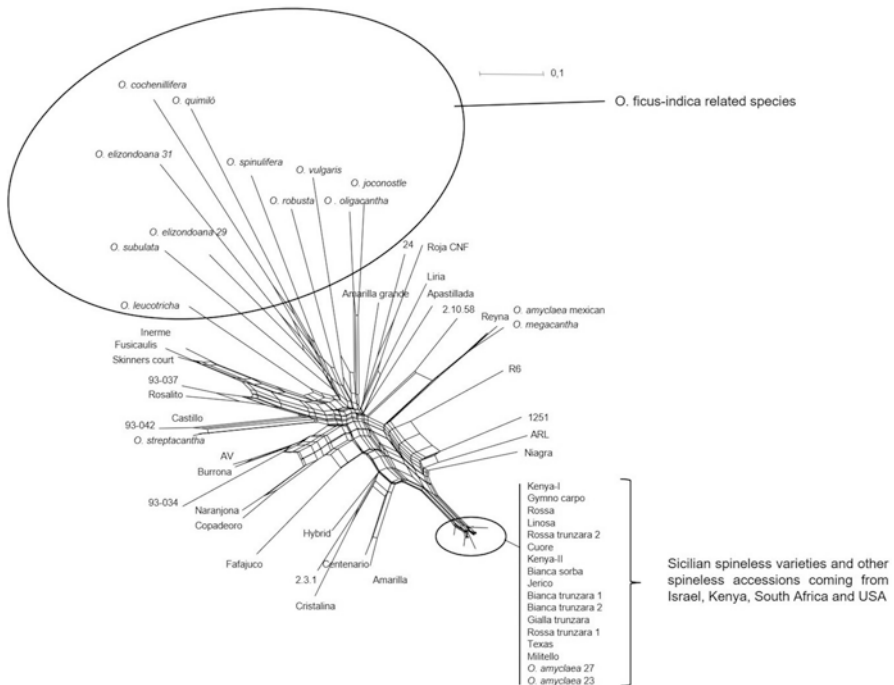
Although molecular analysis could elucidate some taxonomical aspects of the genus and help identify different varieties, the level of genetic diversity among cultivars, wild genotypes, and species related to *O. ficus-indica* is not yet clear. It needs further study with new and more effective analytical tools.

Caruso et al. (2010), using SSRs markers, analyzed 62 genotypes belonging to 16 *Opuntia* species consisted of cultivars, wild genotypes, and cactus pear-related species. In particular, six highly polymorphic simple sequence repeats (SSR) developed by Helsen et al. (2009) and two expressed sequence tag (EST)-SSR loci (developed from *O. streptacantha* EST sequences available in the NCBI database) were used to fingerprint *Opuntia* genotypes of Mexican, Israeli and Sicilian origin including artificial hybrids, and cactus pear-related species belonging to a germplasm collection from the Hebrew University of Jerusalem (Rehovot, Israel).

A clear separation between cultivated accessions and cactus pear-related species was documented. The relationship discovered among the genotypes, investigated by a model-based Bayesian analysis to evaluate the *Opuntias*' genetic structuring, confirms their complex evolution process, in which polyploidization, hybridization, and recombination have played a role. Some spineless genotypes, diverged from the

main group of spineless cultivars; these results suggest that spinescence might have been developed multiple times during the evolution of the genus, and might have been selected from different populations, corroborating the hypothesis of Griffith (2004), who suggested that the spineless varieties might have originated from different ancestors. According to the results of NeighborNet analysis, most of the *O. ficus indica*-related species (*O. cochenillifera*, *O. elizondoana*, *O. oligacantha*, *O. quimilo*, *O. robusta*, *O. spinulifera*, *O. subulata*, *O. vulgaris*, *O. joconostle*) clustered together and were separated from the cultivated varieties (Fig. 7.5). Other *O. ficus-indica* samples did not cluster separately from other species (*O. amyclaea*, *O. megacantha*, *O. streptacantha*, *O. fuscicaulis*, and *O. albicarpa*), highlighting the low consistency of the current taxonomical position with the genetic patterns. In subsequent years, the same Italian group analyzed, using SSR markers, 31 genotypes selected in Brazil for forage production, including *Opuntia ficus-indica* in comparison with related species, covering at least 10 different species, to establish their genetic variability and to elucidate phylogenetic relationships among cultivated genotypes and related species (Las Casas et al., 2017).

Most of the accessions selected in Brazil to be used as forage crops exhibit a narrow genetic variation level indicating that they probably have common ancestors



**Fig. 7.5** NeighborNet tree of SSR data obtained from 62 *Opuntia* genotypes. This analysis supports the hypothesis that *O. ficus-indica* consists of a group of different unrelated clones derived from different parental species and selected for different agronomical features such as the lack of spines and the fleshy fruit. (Modified from Caruso et al., 2010)

and clustered separately from two reference *O. ficus indica* genotypes used for fruit production in the Mediterranean. Finally, once again, the study confirmed the fact that the taxonomical classification of most of the accessions is not congruent with the observed patterns of genetic diversity.

For a better knowledge of the phylogenesis of the cactus family, further investigation of molecular diversity among and within cultivated species and their relationships with putative ancestors has been performed with chloroplast DNA (Nyffeler, 2002; Butterworth & Wallace, 2004; Bonatelli et al., 2013). Based on chloroplast and mitochondrial DNA, cytoplasmic molecular markers are not interested in recombination and showed maternal haploid inheritance. This kind of marker can also overcome polyploid phylogenetics problems, determined by the multiple gene copies (Griffith & Porter, 2009).

Universal chloroplast microsatellites (cpSSR) were used in combination with AFLP markers in the already cited paper by Labra et al. (2003) to characterize the genetic relationship among several *Opuntia* species. Interestingly *O. spinulifera*, *O. undulata*, *O. megacantha*, *O. amyclaea*, *O. robusta*, and *O. ficus-indica* shared the same cpSSR profile.

To reconstruct the phylogeny within *Opuntia* species, many researchers used sequences of plastid intergenic spacers and genes, such as *trnK-matK*, *atpB-rbcL*, *ndhF-rpl32*, *psbJ-petA*, *trnL-trnF*, and *matK-ycf1* (Griffith & Porter, 2009; Barcenas et al., 2011; Majure et al., 2012; Realini et al., 2015). Recently, new extranuclear molecular markers have been identified by the database mining of whole genome sequences (Lemmon & Lemmon, 2013). However, by now, for developing a specific extranuclear marker for *Opuntias*, the only resource consists of sequences from a few genes that are available in the database (Majure et al., 2012; Realini et al., 2015). Las Casas et al. (2018) designed for the first time, specific chloroplast microsatellites for the *Opuntia* genus starting from alignment of DNA sequences. They combined capillary electrophoresis for newly designed cpSSRs and HRM (High Resolution Melting) for SNV analyses to identify chloroplast and mitochondrial DNA markers in a selected *Opuntia* group to elucidate the maternal origin of the cultivated genotypes and their relationships with wild species. The study evidenced, for the first time, the multiple maternal phylogeny for the fleshy fruit varieties classified as *O. ficus-indica* and revealed as the most common cultivated *Opuntias* for fruit production share a narrow genetic base, while forage genotypes evidenced greater variability. In *Opuntia*, the lack of agreement between nuclear and chloroplast DNA genotyping evidenced in some species could be based on the past or recent occurrence of reticulation and cross-hybridization, introgression, and/or chloroplast capture (Stegemann et al., 2012). However, the evolutionary process needs additional studies before drawing any robust conclusions (Realini et al., 2015). These results agree with those of Srikanth and Whang (2015), who evidenced that the *O. ficus-indica* diffused in Korea is closely related to the related species *O. engelmannii* and *O. ellisiana*, but different from the *O. ficus-indica* samples taken from the GenBank database. The draft chloroplast genome sequencing project of *O. quimilo* reported in NCBI (PRJNA544325), but not still public will



undoubtedly help develop new plastid markers and perform more detailed taxonomic studies.

In the last few years, other *Opuntia* sequences were made available in public databases. Specifically, a resource of *O. ficus-indica* gene sequences from seedlings, cladodes, floral buds, floral organs, and fruits was deposited in NCBI (Accession no. PRJNA264306) (Mallona et al., 2011). This represents a source of new potential codominant markers. To date, no whole-genome projects are reported in public databases. It is very likely that, with the drop in sequencing cost, genomic data will be available for the scientific community in the next few years. This will undoubtedly facilitate the development of more sets of *Opuntia* specific markers that could be useful for taxonomy and for genotyping the existing germplasm collections.

## 5 Conclusions

Cactus pear (*Opuntia* spp.) is well considered as a choice crop or even a miracle crop (Inglese et al., 2017), given its peculiar morpho-physiological features, mainly the high water use efficiency and drought tolerance, but also the extensive range of uses. These include its use as fruit, forage, or vegetable crop, the utilization as living fences in dry regions, the contribution to erosion control, and the biomass production in arid areas (Fig. 7.6) (Mondragòn-Jacobo & Chessa, 2013).

Despite this high potential, genetics and breeding of these species have been so far not deeply developed due to some criticisms in its biology (ploidy level) and insufficient knowledge, management, and use of genetic resources. Generally speaking, *Opuntia* germplasm exploitation for breeding purposes is a complex task for the high rate of apomictic seedlings and the very long juvenile period (up to 4–7 years). This reduces the efficiency of generating new variability.

If the effort for an intense genetic improvement activity did not seem necessary in the past years, in particular for fruit varieties, in the future, the occurring climate changes could increase interest in Opuntias. In particular, it may be more and more useful to exploit the high efficiency of water use and all the aspects that make them useful species in the soil defense from desertification. These physiological traits of *Opuntia* species, combined with their destination as a forage crop, can make them particularly important to maintain adequate levels of well-being for the populations of the driest areas of the planet.

In this perspective, germplasm resources are destined to play a crucial role for all future applications of the cactus pear so that the correct classification of accessions along with precise phenotyping is of paramount importance to realize the full potential of this valuable plant resource, for the set-up of precise and efficient breeding schemes based on the efficient identification of useful traits including drought and frost resistance, indigenous and foreign pests and diseases tolerance, and improved nutritional content. Germplasm collection and characterization are a time and cost-expensive process, so a rational approach to identifying the real, useful genetic variability, according to the different uses of the plant to be developed, is needed.



**Fig. 7.6** Intensive plantation of *Opuntia ficus-indica* for biomass production

In *Opuntia*, several studies carried out with different molecular markers have evidenced the inconsistency of the current taxonomy classification of most of the species and cultivar of *O. ficus-indica* and tried to explain the reasons for this problem (Valadez-Moctezuma et al., 2014). In particular, evidence supporting the hybrid origin of some *Opuntia* species and the introgression (introgressive hybridization) by repeated backcrossing of a hybrid with one of its parent species have been documented (Griffith, 2003).

In *O. ficus-indica*, a polyphyletic origin has been assumed by Griffith (2004) and then supported by Caruso et al. (2010) using microsatellites. These evidences should be considered when acquiring, collecting, conserving, and evaluating genotypes in different areas. Another factor generating confusion among accessions is the high level of phenotypic variability, under different agroclimatic conditions, in different cultivation areas so that for a thorough investigation, different experimental sites are needed with an increase in costs of research.

In such a picture, molecular tools can exert an essential role in order to identify homonymies and synonymies in the collections, so reducing the number of accessions to be kept into the collection, to identify new and rare alleles to be preserved from genetic erosion, and to set up core collections of the existing genetic variability. Core collections are smaller in size and therefore easier to be kept and managed and contain specific genotypes useful for breeding. Similar approaches have been recently proposed for the carob tree, an endangered multipurpose minor fruit tree species (Di Guardo et al., 2019).

The set-up of genetic resources collection and their management requires significant efforts. One of the biggest problems is represented by the frequent redundancy of the accessions, increasing the number of plants to be maintained. Molecular marker approaches can limit this problem allowing precise identification of the collected items based on the genetic characteristics. The share adoption of robust phenotyping protocols, including the preliminary agronomical evaluation and the genotyping with reliable molecular methods, is highly advisable for all the germplasm banks, core collections, and cultivar trials currently diffused in all the major cactus-pear-producing countries.

The definition of adequate passport data, complemented with genetic data, would optimize the conservation programs' efficiency, and facilitate access to final users' materials and information. In the future, these programs would also benefit from the data of a whole genome sequencing approach, similar to what was observed for an increasing number of species. An integrated approach of molecular-based characterization and the adoption of accurate large-scale phenotyping of existing germplasm collections could lead to functional management of genetic resources for their sustainable conservation and plan future breeding strategies for improved genotypes to be cultivated in distinct areas for different purposes. A common platform is needed to facilitate the exchange of information, and its set-up would deserve attention from all the potential users.

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# Chapter 8

## Genetic Diversity and Ecotypes of *Opuntia* spp.



Ahmad A. Omar, Abdelaleim I. ElSayed, and Azza H. Mohamed

**Abstract** The genus *Opuntia* belongs to the family Cactaceae, subfamily Opuntioideae, tribe Opuntieae. Many *Opuntia* is found on roadsides and routes due to usual ruderal behavior and it is planted for nourishment fruit production and border formation. The plant genetic background of any species plays an essential role in the improvement of plants by enriching the germplasm with a big pool of genetic variation for the breeders to create new cultivars. For many years *Opuntia* taxonomy was based on the morphological description and subsequently enriched with biochemical, physiological, and cytogenetic parameters. Molecular markers are widely used and overcome problems associated with genetic variation, genome mapping, phylogenetic, and evolutionary studies. *Opuntia* spp. has some individualities regarding molecular marker investigation, such as polysaccharides content and secondary metabolites, which makes DNA isolation extremely difficult. Besides, the ploidy level among *Opuntia* spp. cause several problems in the analysis of codominant markers. The polyploidy level in *Opuntia* can range from triploid ( $2n = 3x = 33$ ) to octoploid ( $2n = 8x = 88$ ). The ploidy level of *Opuntia* spp. depends on the population's origin. *Opuntia* spp. cultivated in regions that differ from the origin present a lower genetic diversity than that of the areas of origin. Due to the presence of cleistogamy (self-fertilization) and polyembryony and the lack of rain and low temperature, seed germination is difficult, which makes the extent of genetic diversity limited. To increase the genetic variability among *Opuntia* populations, it requires

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introducing germplasm and landraces from the center of origin and domestication besides adding different genotypes from other regions.

**Keywords** Origin of *Opuntia* spp. · Phenotypic diversity · Genome size · Ploidy levels · Apomixis · Molecular marker

## Abbreviations

cpSSR	Chloroplast simple sequence repeats
GA	Gibberellic acid
ISSR	Inter-simple sequence repeats
nuSSR	Nuclear microsatellite
PCR	Polymerase chain reaction
QTL	Quantitative trait locus
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeats
UPGMA	Unweighted pair group method with arithmetic mean

## 1 Introduction

The *Opuntia* spp. (Cactaceae, Opuntioideae), is a xerophytic plant native to Mexico (Reyes-Aguero et al., 2005; Russell & Felker, 1987). It grows in many regions worldwide, such as; North and South America, Africa, India, Australia, and the Mediterranean countries (Piga, 2004). The species *O. ficus indica* is native to Mexico, where it was domesticated by the ancient Mexicans (Griffith, 2004; Kiesling, 1998). After discovering America, *O. ficus indica* was introduced into Spain by sailors because of its anti-scurvy properties. Afterwards, it was introduced to other parts of the world, particularly to the Mediterranean region (Kiesling, 1998). Generally, the *Opuntia* spp. are well adapted to grow in arid and semi-arid environments and tolerated poor soils (Gallegos-Vazquez et al., 2012). Furthermore, *Opuntia* spp. have been domesticated a long time ago throughout arid and semi-arid regions and constituted until today an essential crop in many countries' agricultural economy (Griffith, 2004). Particularly, *Opuntia* spp. is an alternative crop for the Mediterranean region's economy because of the increase in temperatures and the lack of rain during summer in this region. In many countries, *Opuntia* spp. was intensively cultivated for commercial purposes. *Opuntia* crop is popular in some countries due to its attractive taste, nutritional value, and effects on human health (Díaz et al., 2017; Zakyntinos & Varzakas, 2016). Its economic and ecological importance rises from the fact that it can be used as a forage crop for cattle and other

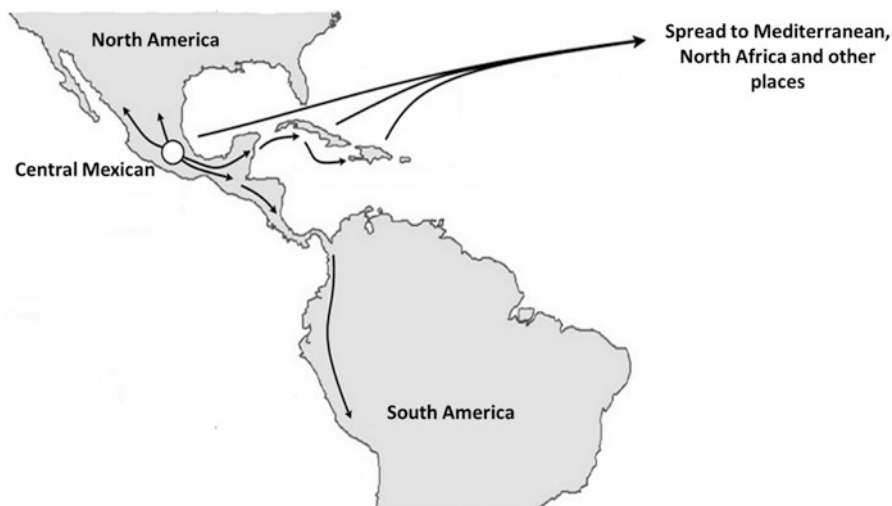
animals, especially when there is a shortage of fresh forage due to drought (Anaya-Perez, 2001; Mondragón-Jacobo & Pérez-Gonzalez, 2001; Nefzaoui & Ben Salem, 2001; Viguera & Portillo, 2001) or as a medicinal plant (Griffith, 2004).

The edible parts of *Opuntia* plants are known as cladodes, pads, nopales, or pen-cas, as well as the tender young part of the cactus stem consumed as a vegetable in salads. Mexico and Italy are the main producer and consumers countries. From the approximately 590,000 ha cultivated worldwide, Mexico accounts for 70% and Italy for 3.3%. Under optimal conditions, Mexico's annual production can reach 350,000 tons (Reyes-Agüero & Rivera, 2011). The cultivated *Opuntia* spp. are grown in at least 30 countries worldwide (Díaz et al., 2017).

The taxonomic classification of the *Opuntia* genus has been reported as a complex, which explains many reports of misclassification of *Opuntia* spp. (Samah et al., 2016). Continuous morphological variation and limited morphological descriptions for cultivar discrimination are the most difficult obstacles to achieve a stable classification (Caruso et al., 2010; Labra et al., 2003; Samah et al., 2016; Valadez-Moctezuma et al., 2014). Thus, the difficulties in morphological interpretation have led to the publication of many binomials, many of which are synonyms, homonyms, or false attributions (Gibson & Nobel, 1986).

## 2 Geographical Origin of *Opuntia* spp. and History of the Movement

The genus *Opuntia* in the Cactaceae family (subfamily Opuntioideae), which included several species, widespread in different areas around the world such as North and South America, the Mediterranean basin, Middle-East, South Africa, India, Thailand, and Australia (Griffith, 2004). The genus *Opuntia* is one of the major recognized genus of the Opuntioideae, which includes over 181 species and 10 naturally occurring hybrids (Anderson, 2001). Several species of genus *Opuntia* become cultivated, such as *O. robusta*, *O. megacantha*, *O. hyptiacantha*, *O. cochenillifera*, *O. streptacantha*, *O. albicarpa*, and *O. amyclaea*, (Kiesling, 1998; Mondragón-Jacobo & Pimienta-Barrios, 2001; Pimienta, 1990; Scheinvar, 1995). The most widespread and economically important species is *O. ficus indica*. This species, commonly called Indian fig, cactus pear, prickly pear, and barbary fig, which probably domesticated in central Mexico for 9000 years and spread in several warm regions of the world by European travelers beginning in the late fifteenth century (Griffith, 2004; Kiesling, 1998). *O. ficus-indica* specie was certainly known at the beginning of the sixteenth century (Casas & Barbera, 2002). Further, *O. ficus-indica* fruits and shoots were also reportedly consumed by the Maya of southeastern Mexico (Coe, 2015). There is also some evidence for the use of *O. ficus-indica* by the Nazca of Peru, placing these plants in South America at a very early date (Sejuro, 1990). Figure 8.1 explains how the *O. ficus-indica* species was found early in central Mexico and the cultivated plants then have been spread through trade



**Fig. 8.1** A biogeographic model of dispersal of *Opuntia ficus-indica*. From central Mexico, the ancestors of *O. ficus-indica* were selected from arborescent, fleshy-fruited taxa (one or more species such as *O. leucotricha*, *O. hyptiacantha*, *O. megacantha*, and *O. streptacantha*). The cultivated plants then spread through trade throughout Mesoamerica and the Caribbean and possibly into South America. European travelers then spread these plants into Mediterranean Europe and North Africa and subsequently throughout the world's arid and semi-arid regions. The graph has been adapted and modified from Griffith (2004)

throughout Mesoamerica and the Caribbean and possibly into South America. Then the European travelers spread these plants into Mediterranean Europe and North Africa and subsequently throughout arid and semi-arid regions (Griffith, 2004). The biogeographic and evolutionary origins of *O. ficus-indica* species have been obscured through ancient and widespread cultivation and naturalization. Griffith (2004) studied the origin of *O. ficus-indica* species through Bayesian phylogenetic analyses of the internal transcribed spacer DNA sequences. The results of this study revealed that *O. ficus-indica* is a close relative of a group of arborescent, fleshy-fruited prickly pears from central and southern Mexico; that the center of domestication for this species is in central Mexico; and that the taxonomic concept of *O. ficus-indica* may include clones derived from multiple lineages and therefore be polyphyletic.

### 3 Morphological Taxonomy (Phenotypic Diversity) of *Opuntia* spp.

Based on vegetative and floral morphology, four major subfamilies have been classified in the Cactaceae family: Pereskioideae, Cactoideae, Maihuenioideae, and Opuntioideae (Anderson, 2001; Barthlott & Hunt, 1993; Gibson & Nobel, 1986).

*Opuntia* is one of the major recognized genus of the Opuntioideae, including over 181 species and 10 naturally occurring hybrids (Anderson, 2001). Amani et al. (2019) studied the morphological characters of four *Opuntia* genus species (*Opuntia tomentosa*, *Opuntia ficus-indica*, *Opuntia undulata*, and *Opuntia engelmannii*). As mentioned in Table 8.1, quantitative and qualitative parameters showed the most discriminant traits allowing separating among the four studied species. Small red-pink flowers and dark green cladodes with velvety texture are the important apomorphic traits found for the species “*Opuntia tomentosa*”. Furthermore, *Opuntia undulata* seems to be one species with many apomorphic characters; the light green color of cladodes, light yellow flowers, and purple fruits are observed only in this species. The results also revealed a very high level of morphological variability and a remarkable similarity between *O. ficus-indica* and *O. engelmannii* (Amani et al., 2019). Gallegos-Vazquez et al. (2012) found that qualitative variables such as flesh and peel color proved useful to distinguish among potentially the 21 redundant accessions collected from Mexico. Gutiérrez-Acosta et al. (2000) mentioned that some other qualitative traits, such as fruit dimensions, are useful in separating cactus pear accessions (Gallegos-Vazquez et al., 2012).

**Table 8.1** Quantitative and qualitative characters of four *Opuntia* spp. to discriminant morphological variability.

Quantitative and qualitative parameters	<i>Opuntia</i> spp.			
	<i>O. ficus-indica</i>	<i>O. engelmannii</i>	<i>O. undulata</i>	<i>O. tomentosa</i>
Number of seeds per fruit	Very low seeds number	Considerable number of seeds	Very low seeds number	Very low seeds number
Number of areolas/cladode	Very low number of areolas/cladodes	Considerable number of areolas/cladodes	Very low number of areolas/cladodes	Considerable number of areolas/cladodes
Fruit weight	Small fruits	Medium fruits	Big fruits	Small fruits
Cladode shape and color	Green cladodes ovoid, round or elongated cladodes Smooth cladodes	Green cladodes Ovoid, round or elongated cladodes Smooth cladodes	Light green cladodes Oblong curved cladodes Smooth cladodes	Dark green cladodes Ovoid, round or elongated cladodes Cladodes with a velvety texture
Flowers color	Yellow, light orange, orange, red-orange hermaphrodite flowers	Yellow, light orange, orange, red-orange hermaphrodite flowers	Light yellow hermaphrodite flowers	Red-pink hermaphrodite flowers
Fruit color/pulp color	Green, yellow, greenish-yellow, orange, or red fruits	Green, yellow, greenish-yellow, orange, or red fruits	Purple fruits	Green, yellow, greenish-yellow, orange, or red fruits

*O. ficus indica* reproduces sexually and propagates vegetative (Reyes-Agüero et al., 2005), where outcrossing (allogamous) is common among cacti family (Pimienta & del-Castillo, 2002). This phenomenon may lead to different genetic diversity among this species. Generally, the cactus is present in different climatic ecosystems in the world. The cactus family's highest diversity degree was found in Mexico, followed by Brazil, Bolivia, and Peru (Ortega-Baes et al., 2010). This diversity was positively associated with environmental factors such as temperature and precipitation (Mourelle & Ezcurra, 1997). The domestication process of *Opuntia* was begun by producing spineless cladodes with large, sweet fruits. The partial or total absence of the spine is the main diagnostic character of *O. ficus indica* (Reyes-Agüero et al., 2005), where it has been mistakenly related to *O. ameclya*, *O. megacantha*, and *O. streptacantha* (Kiesling, 1998; Labra et al., 2003). *O. ficus indica* was considered as a synonym of *O. megacantha* since the presence or absence of spines is insufficient for separating them (Benson & Walkington, 1965). Based on the combination of the differential vegetative and reproductive characters, Reyes-Agüero et al. (2005) considered that *O. ficus indica* constitutes a taxonomic entity that differs from *O. megacantha* and *O. streptacantha*. Moreover, (Kiesling, 1998) suggested that the spiny and spineless specimens are only forms of *O. ficus indica*.

The dominant color for flowers in *Opuntia* spp. is yellow, but there are also pink, orange, red, white, purple, mottled flowers (Anderson, 2001). Hermaphrodite flowers are the most common (Anderson, 2001; Gibson & Nobel, 1986). Stamens are numerous, being 265 in *O. polyacantha*, 358 in *O. phaeacantha* (Osborn et al., 1988), 450 in *O. viridirubra*, and 598 in *O. brunneogemmia* (Schlindwein & Wittmann, 1997). Stamens are generally yellow or green (Grant et al., 1979) with a circular or spiral arrangement around the style (Boke, 1980). In some cases, the stamens closest to the style are short and successively grow longer, with the longest occurring close to the tepals (Grant & Grant, 1981; Schlindwein & Wittmann, 1997).

#### 4 Genome Size and Ploidy Levels in *Opuntia* spp.

Polyploidy is a usual situation throughout the tribe Opuntieae. Diploids ( $2n = 2x = 22$ ) are uncommon in this tribe, according to the reported chromosome counts (Majure et al., 2012b). Polyploid taxa within *Opuntia* spp. range from triploid ( $2n = 3x = 33$ ) to octoploid ( $2n = 8x = 88$ ), and many species have multiple ploidy levels (Majure et al., 2012a; Pinkava, 2002). The ploidy level of the studied species is unclear since the information from the literature lacks concordance, particularly in the case of *O. dillenii* ( $2n = 12, 22, 26, 36, 40, 44, 66$ ), *O. elata* ( $2n = 22, 44$ ) and *O. robusta* ( $2n = 22, 44, 66, 88$ ) (Majure et al., 2012b). The ploidy level of cultivated *O. ficus-indica* populations is  $2n = 88$  (Segura et al., 2007). However, penta, hexa, hepta, and diploid levels were also reported in these species, depending on the population origin (Majure et al., 2012a).

Polyploidy and asexual reproduction of zygotes and embryos have long been correlated (Stebbins, 1980). Different ploidy levels occur in the Cactaceae, and it has been suggested that both polyploidy and hybridization events have led to speciation in this family (Cota & Philbrick, 1994; Pinkava et al., 1985). Relative to the basic chromosome number for the family and *Opuntia*,  $x = 11$ , *O. spinosissima* is considered a hexaploid species ( $2n = 66$ ; Cota, unpublished data; (Pinkava et al., 1985). According to J. H. Cota (personal communication), the chromosomes are very small and morphologically uniform in shape and size. Additionally, Cota suggested that this taxon is a consequence of a recent polyploidization event; therefore, low genetic diversity is expected. Electrophoretic data showed that this population is genetically monomorphic for 12 of 13 loci, and at the remaining locus (malate dehydrogenase-1), all individuals are heterozygous, suggesting this genotype is fixed (Hamrick & Godt, 1997). Evidence implies that these 13 extant plants are clones from a single lineage.

The 2C-DNA amount of the *Opuntia* spp. analyzed ranged from 4.17 pg for *O. incarnadilla* to 6.53 pg for the diploid *O. heliabravoana* (Segura et al., 2007). Four ploidy levels, diploid, tetraploid, hexaploid, and octoploid, were identified among the 23 species of *Opuntia* (Table 8.2). Of the 18 species from the series *Streptacanthae* as determined by Estrada-Galván et al. (2000), 15 were octoploid, 2 were hexaploids (*O. incarnadilla* and *O. matudae*), and 1 (*O. elizondoana*) produced tetraploid values (Table 8.2).

The mean 2C genome of the group of principal edible species of *Opuntia* analyzed here is 5.05 pg. In comparison to 2C-values for the cacti by Bennett and Leitch (2005), the mean of *Opuntia* spp. analyzed here exceeded those of *Escobaria bella* Britton et Rose ( $2C = 3.05$  pg), *Pseudolobivia* sp. ( $2C = 3.25$  pg), *Borzicactus aurivillus* K. Schum. ( $2C = 3.35$  pg), *Cleistocactus smaragdifolius* (F.A.C. Weber) Speg. ( $2C = 3.35$  pg), *Aporocactus flagelliformis* Lem. ( $2C = 3.80$  pg), and *Trichocereus werdermannianus* Backeb. ( $2C = 3.90$  pg), but less than most of *Mammillaria* species reported or *Weberbauerocereus winterianus* Anthony ( $2C = 14.20$  pg). Variations of ploidy levels estimated for this *Opuntia* spp. are consistent with their assignment to series, except *O. heliabravoana*, *O. elizondoana*, *O. matudae*, and *O. zamudioi*.

Opportunities are already available to test the value of *Opuntia* genome size as a predictor of environment and evolution responses. The full value of nuclear DNA amounts will likely be realized only when these determinations can be applied in association with other measurable plant traits (Grime, 1998; Otto & Whitton, 2000). Variations in chromosome size and subsequent meiotic abnormalities have disrupted breeding programs. *O. ficus-indica* x *O. robusta* ssp. *robusta* has been used as the basis of recombinant populations, and such variation could explain some of the segregationally disturbances (Cota & Philbrick, 1994; Michael Powell & Weedon, 2001; Pinkava et al., 1973, 1985).

**Table 8.2** Estimated DNA amounts, species, subspecies and varieties groups and ploidy levels as determined for *Opuntia* spp. by flow cytometry, according to Segura et al. (2007).

Species	2C-DNA amount estimated (pg)	Interpretation (ploidy level)
<i>O. leucotricha</i>	5.71	4X
<i>O. spinulifera</i>	5.51	4X
<i>O. oligacantha</i>	5.33	6X
<i>O. Zamudioi</i>	4.35	8X
<i>O. lasiacantha</i>	4.88	8X
<i>O. hyptiacantha</i>	4.84	8X
<i>O. streptacantha</i> ssp. <i>streptacantha</i>	4.64	8X
<i>O. streptacantha</i> ssp. <i>aguirrana</i>	4.43	8X
<i>O. megacantha</i>	5.01	8X
<i>O. joconostle</i>	4.70	8X
<i>O. ficus-indica</i>	4.90	8X
<i>O. albicarpa</i>	4.80	8X
<i>O. amarilla</i>	4.84	8X
<i>O. chavena</i>	4.70	8X
<i>O. cochineria</i>	5.10	8X
<i>O. incarnadilla</i>	4.17	6X
<i>O. fuliginosa</i>	4.64	8X
<i>O. pachona</i>	4.70	8X
<i>O. matudae</i>	5.25	6X
<i>O. cretochaeta</i>	4.35	8X
<i>O. rzedowskii</i>	4.77	8X
<i>O. elizondoana</i>	5.29	4X
<i>O. heliabravoana</i>	6.53	2X
<i>O. robusta</i> var. <i>robusta</i>	4.98	8X
<i>O. robusta</i> var. <i>larreyi</i>	5.96	4X
<i>O. robusta</i> var. <i>guerrana</i>	5.05	8X
<i>Cylindropuntia imbricata</i>	6.92	2X

## 5 Apomixis in *Opuntia* spp.

Apomixis frequently occurs in *Opuntia* (Mondragon-Jacobo & Perez-Gonzalez, 1996; Pimienta 1990). Apomixis is the production of seeds without previous fertilization (Mondragón-Jacobo & Bordelon, 2002). In *Opuntia*, the most common method is the development of adventitious embryos from nucellar tissue (sporofitic agamospermy) (Garcia-Aguilar & Pimienta-Barrios, 1996; Mondragón-Jacobo & Pimienta-Barrios, 2001; Vélez-Gutierrez & Rodríguez-Garay, 1996) or like in *O. streptacantha*, embryos can be developed from an unfertilized egg (diplospory-parthenogenesis) (Garcia-Aguilar & Pimienta-Barrios, 1996). *O. streptacantha*

flowers emasculated and isolated from exogenous pollination produce fruits with seeds. This result has been interpreted as evidence of apomixis (Pimienta & del-Castillo, 2002; Trujillo Argueta et al., 1986). Polyembryony has also been considered as proof of apomixis (Mondragón & Pimienta, 1995). Thus, apomixis is said to occur commonly in members of *Opuntia*. For example, 20 of the 23 most important fruit cultivars of *Opuntia* in the San Luis Potosí and Zacatecas highlands form polyembryonic seeds (Pimienta & del-Castillo, 2002), although only 3–4% of seeds per fruit are polyembryonic (Mondragón & Pimienta, 1995). Apomixis is more frequent in xenogamic cultivars (Mondragón-Jacobo & Pimienta-Barrios, 2001). Polyembryony is common in wild populations of *O. robusta*, *O. cochineria*, *O. leucotricha*, *O. rastrera*, *O. streptacantha* (Trujillo Argueta et al., 1986), *O. joconostle* (Sánchez, 1997), and *O. stricta* (Reinhardt et al., 1999). Thirty-three percent biembryony, 13% tri-embryony, and 4% tetra-embryony have been reported for *O. ficus-indica* (Nieddu & Chessa, 1996). Seedless fruits have been obtained experimentally by inducing male sterility with a chemical gameticide and gibberellic acid (GA) (Aguilar & Chávez, 1995; Gil & Espinoza, 1980). Emasculated flowers with an application of GA develop seedless normal-sized fruits. The most efficient treatments were: (1) a single application of 500 mL L<sup>-1</sup> GA during anthesis and (2) the application of 100 mL L<sup>-1</sup> GA 22 and 42 days after anthesis (Aguilar & Chávez, 1995). GA inhibits seed development and induces fruit growth, causes fruit development with a thin peel, little pulp, and a low total content of soluble solids (Nerd & Mizrahi, 1994). Since epidermal cells in the funicular cover will not differentiate without fertilization, Rosas and Pimienta (1986) stated that *Opuntia* could not produce parthenogenetic fruits.

*O. monacantha* is not an apomictic species. According to Reyes-Agüero et al. (2006), apomixis is rare in *Opuntia*, although it has been reported in *O. aurantiaca* Lindl., *O. dillenii* Haw., *O. leucantha* Link. & Otto, and *O. tortispina* Engelm. & J. M. Bigelow. In these species, the apomictic seeds are almost always of nuclear origin (sporophytic agamospermy) and do not demonstrate abnormalities (Mondragón-Jacobo & Bordelon, 2002). On the other hand, Osborn et al. (1988) reported that the seeds of *O. polyacantha* Haw. and *O. phaeacantha* Engelm. that arose through xenogamy demonstrated greater viability than those produced by other forms of pollination. The same situation appears to apply to *O. monacantha*, which demonstrated high seed production levels and germination arising from natural pollination regimes and manual crosspollination experiments. Additionally, most of the fruits generated from self-pollination experiments aborted, and these abortion rates were higher than those observed in cross-pollination. These results suggest that the abortion of fruits formed through spontaneous or manual self-pollination may represent situations of delayed incompatibility or endogamic depression, suggesting the predominance of endogenous pollen flux between flowers of different clones.



## 6 Techniques to Study the Genetic Diversity of *Opuntia* spp.

Molecular markers play an important role in the improvement of wild and cultivated plants. Isozymes and restriction fragment length polymorphism (RFLP) are molecular markers that have been largely used as reliable markers for plant genetic analyses (Wang et al. 1992, 1998). PCR-based techniques provide DNA markers scattered throughout the genome which are easier to analyze (Vos et al., 1995). Mainly, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) have been widely used for accession classification, cultivar identification, cereals and vegetables, and for diversity estimation or genetic variability of complex species too (Bendhifi et al., 2013; Shilpha et al., 2013; Xiao et al., 2004). Since these markers are very useful because of their simplicity, low cost and performance capacity (Waugh & Powell, 1992), particularly ISSR analysis, it continues to be used as an alternative tool for SSR diversity studies since they are repeatable (Zietkiewicz et al., 1994).

In the last decade, molecular markers have been applied to complement morphological characters in determining genetic diversity to address the taxonomic uncertainty regarding the delineation of the various species within *Opuntia* (Erre et al., 2010). Different molecular methods have been proven to be used in classifying the different species of *the Opuntia* plant (Gordon & Kubisiak, 1998; Labra et al., 2003; Wang et al., 1998). Among the molecular markers, RAPD, Chloroplast simple Sequence Repeats (cpSSR) (Chessa et al., 2008), and Simple Sequence Repeats (SSR) (Helsen et al., 2009) have been used to identify and characterize genetic diversity in *Opuntia* spp. Microsatellites have been used in germplasm diversity evaluation, phylogenetic and evolutionary studies, and genome mapping (Kalia et al., 2011). Microsatellites, also known as simple sequence repeats (SSRs), are non-coding, repetitive DNA regions consisting of tandem repeated small motifs (1–6 bp); they are present throughout the genome of an individual, both in coding and non-coding regions. In recent years, microsatellites have been demonstrated to have many important biological functions (e.g., the regulation of chromatin organization, DNA metabolic processes, gene activity, and RNA structure) (van der Knaap & Verrijzer, 2016).

In comparison to other molecular markers, microsatellites are the most informative due to their reliability and abundant multiallelic forms. They exhibit higher mutation rates than the rest of the genome (Gao et al., 2016) and can be easily analyzed using PCR-based methods, including fluorescent automated genotyping and multiplexing. Therefore, SSR markers have been the preferential choice for various applications, such as variety identification, genetic diversity evaluation, phylogenetic relationship analysis, genetic map construction, linkage/association mapping of gene/QTL, marker-assisted selection, and comparative mapping (Shilpha et al., 2013). Labra et al. (2003) reported that the combination of cpSSR and AFLP markers provides a quantitative estimation of genetic relationships among several *Opuntia* spp.

Molecular markers represent reliable tools for the characterization and genetic variability of the *Opuntia* varieties since the environment does not influence them. (Valadez-Moctezuma et al., 2015) assessed the genetic variation of 52 *Opuntia* cultivars using marker tools (RAPD and ISSR). This analysis revealed that *O. ficus-indica*, *O. albicarpa*, and *O. megacantha* species exhibited high genomic variation, while varieties of *O. xocostle*, *O. robusta*, and *O. streptacantha* showed a higher level of association.

Other ambiguities in the taxonomic classification of *Opuntia* spp. emerged in a study that used microsatellite polymorphisms to try to discriminate between two morphologically distinct *O. echios* botanical varieties (echios and gigantea) native to the Galapagos islands (Helsen et al., 2009). Once again, the authors highlighted that molecular data did not support the current taxonomic differentiation between these taxa. Although these studies clarified some taxonomical aspects of the genus and were useful for cultivar fingerprinting, there is still a lack of knowledge regarding the level of genetic diversity among the most diffused cultivated genotypes throughout the world and the diversity of cultivars, wild genotypes, and species related to *O. ficus indica*. Garcia-Zambrano et al. (2009) used microsatellite markers (SSR) to investigate the genetic diversity among *O. ficus indica* cultivated varieties and some related species. They reported that SSR could analyze a greater number of individuals originating from controlled crosses with different parentals to assess the molecular evolution of polyploidy in *Opuntia* spp.

Additionally, microsatellites may use as fast and reliable techniques to discriminate *Opuntia* apomictic seedlings from zygotic ones (Mondragón-Jacobo & Bordelon, 2002; Reyes-Agüero et al., 2006). Therefore, molecular techniques are the most appropriate tools for assessing the evolution of genetic diversity in *Opuntia* germplasm collections; such analysis should be a prerequisite for planning breeding programs that capture most of the existing variability among prickly pears (Caruso et al., 2010). The use of these molecular markers is strongly suggested to reclassify the cactus pear cultivated accessions, which exhibit a high level of variation regardless of the current taxonomical classification and probably should be classified as the same species, as suggested by Kiesling (1998).

## 7 Genetic Diversity of *Opuntia* spp.

The molecular characterization of populations could assist plant breeders with a better knowledge of the existing genetic variability. Reis et al. (2018) investigated the genetic diversity of 19 Portuguese *Opuntia* spp. populations from the species *O. ficus-indica*, *O. elata*, *O. dillenii*, and *O. robusta* using nuclear microsatellite (nuSSR) markers. Also, they used the Italian cultivars ‘Bianca’, ‘Gialla’ and ‘Rossa’ for comparison purposes. The study of Reis et al. (2018) found no genetic differences between the inermis form, *O. ficus-indica* f. *ficus-indica*, and the rewilded spiny one, *O. ficus-indica* f. *amyclaea*. The UPGMA tree indicated that the clustering pattern was unrelated to the geographical origin. Furthermore, the results from

the same study showed a low level of genetic diversity among the Portuguese populations of *O. ficus-indica*.

Labra et al. (2003) used two molecular techniques: chloroplast simple sequence repeat (cpSSR) and amplified fragment length polymorphism (AFLP) to evaluate the characterization and genetic relationships among 11 *Opuntia* spp. The analysis of cpSSR and AFLP results in *O. ficus-indica* and *O. megacantha* species showed a common genomic constitution and a clear distinction from all other *Opuntia* accessions analyzed. Moreover, based on molecular analysis, morphological traits, and biogeographical distribution, Labra et al. (2003) suggested that *O. ficus-indica* and *O. megacantha* should be considered the same species.

Although most of the *Opuntia* wild species related to cactus pear, they are clustered in separate groups, the genotypes classified as *O. amyclaea*, *O. megacantha*, *O. fuscicaulis*, *O. streptacantha*, and *O. albicarpa* are closely related to the *O. ficus indica* cultivated varieties. The analysis and previous work based on molecular variation (Griffith, 2004; Labra et al., 2003; Wang et al., 1998) support the fact that the present classification of cultivated varieties and wild genotypes based on morphological parameters is misleading. Consequently, molecular tools are the most appropriate tools for assessing the level of genetic diversity in *Opuntia* germplasm collections; such analysis should be a prerequisite for planning breeding programs that capture most of the existing variability among prickly pears. The use of these markers is strongly suggested to reclassify the cactus pear cultivated accessions, which exhibit a high level of variation regardless of the current taxonomical classification and probably should be classified as the same species, as suggested by Kiesling (1998).

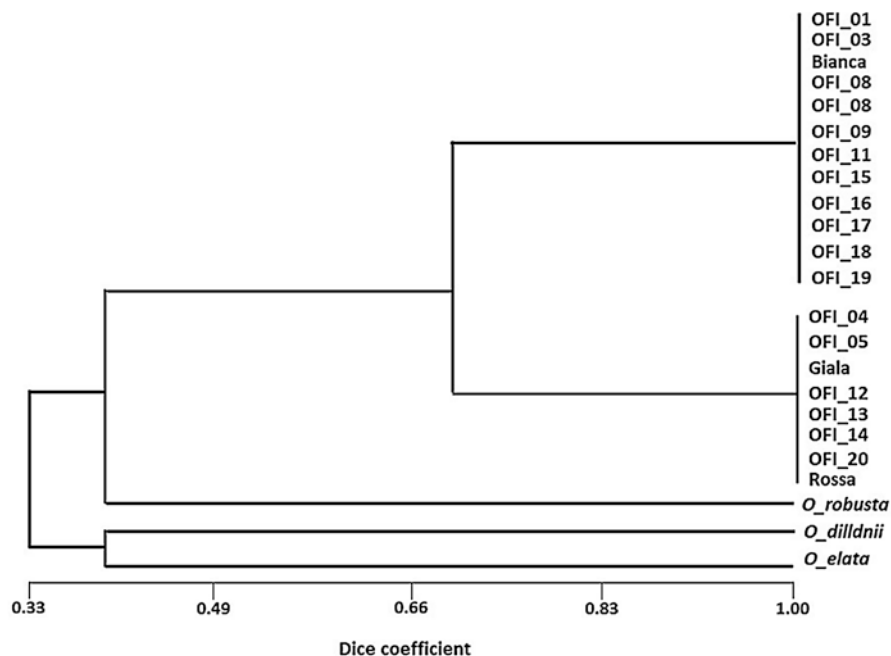
Two important studies (Griffith, 2004; Labra et al., 2003) employed different molecular tools to elucidate taxonomical aspects of the genus, particularly the origin of *O. ficus indica*. Labra et al. (2003) used AFLP to verify the lack of genetic differentiation between *O. ficus indica* and *O. megacantha* populations and suggested that *O. ficus indica* should be considered as a domesticated form of the spiny *O. megacantha*. On the other hand, (Griffith, 2004) considered the species to be a group of different clones, selected for their low number of spines and their fleshy fruits derived from different parentals, most likely from other arborescent opuntias from central and southern Mexico. Most of these DNA-based analyses revealed discrepancies between molecular characterization and classical taxonomical classification.

Caruso et al. (2010) investigated 6 highly polymorphic simple sequence repeats (SSR) and 2 expressed sequence tag (EST)-SSR loci in 62 wild and cultivated genotypes belonging to 16 *Opuntia* spp., which collected from several areas throughout the world. The phylogenetic analyses separated the wild opuntias from the cultivated ones. However, the *O. ficus indica* accessions did not cluster separately from other arborescent cactus pear species, such as *O. amyclaea*, *O. megacantha*, *O. streptacantha*, *O. fuscicaulis*, and *O. albicarpa*, indicating that their current taxonomical classifications do not fit with their genetic variability. In general, the genotypes cultivated in Mexico showed high diversity levels, whereas most of the spineless accessions collected in other countries had a very narrow genetic base.

Furthermore, Browne et al. (2003) evaluated the phylogenetic relationships among 8 of the 14 Galapagos *Opuntia* taxa (240 individuals), using 8 allozyme markers, but found no variation, probably due to conservatism in the allozyme markers they used. Browne et al. (2003) used a larger set of neutral and highly variable microsatellite markers (Goldstein & Schlotterer, 1999) to re-evaluate the genetic variability of two *O. echios* varieties. The ample genetic diversity uncovered by these markers is postulated to allow population structures to be revealed more accurately than by other markers (Liu et al., 2003).

The *Opuntia* genus includes important cultivated species that have been widely studied using different molecular markers, mostly nuclear ones. Although most of the wild *Opuntia* spp. related to cactus species exhibited differences in fruit color, flesh consistency, and thorniness, the results of Las. Casas et al. (2017) showed that genotypes classified as *O. amyclaea*, *O. megacantha*, *O. elizondoana*, *O. streptacantha*, *O. vulgaris*, *O. joconostle*, *O. undulata*, and *O. albicarpa* had the same chlorotype of the *O. ficus-indica* cultivated varieties, clustering into a unique group. More than two-thirds of the analyzed genotypes and 9 species among the 15 species showed the same maternal inheritance, attesting to the narrow genetic variability among the cultivated *Opuntia* genotypes and the above-supposed species. The results were mostly in agreement with those obtained by cpSSR analyses from Labra et al. (2003), who reported that *O. stricta* grouped independently from *O. ficus-indica*, *O. megacantha*, *O. amyclaea*, and *O. undulata*, which have the same chlorotype. However, in the dendrogram, *O. spinulifera* and *O. robusta* segregated separately, as well. Other works confirmed that *O. robusta*, *O. stricta*, *O. leucotricha*, and *O. cochenillifera* had different plastid genealogies (Bárceñas et al., 2011; Majure et al., 2012a; Realini et al., 2015). The maternal inheritance analysis results revealed that genotypes classified as *O. ficus-indica*, *O. albicarpa*, *O. streptacantha*, and *O. megacantha* are closely related and do not show species-specific chlorotype (Fig. 8.2). The result agrees with the previous studies based on nuclear SSRs, ISSRs, and RAPDs (Caruso et al., 2010; Griffith, 2004; Labra et al., 2003; Samah et al., 2016; Valadez-Moctezuma et al., 2015).

Generally, the molecular analysis of plastid genetic variability confirmed previous works based on nuclear marker analysis (Caruso et al., 2010; Griffith, 2004; Labra et al., 2003; Samah et al., 2016; Wang et al., 1998), clearly supporting the fact that *O. amyclaea*, *O. megacantha*, *O. elizondoana*, *O. streptacantha*, *O. vulgaris*, *O. joconostle*, *O. undulata*, and *O. albicarpa* are closely related to *O. ficus-indica*, and weakening the previous taxonomical classification based on the morphological parameters.



**Fig. 8.2** Dendrogram of the 22 *Opuntia* spp. populations obtained from SSR markers based on Dice coefficient and using the unweighted pair group arithmetic mean method (UPGMA) as the clustering method. The data obtained from SSR markers and phylogenetic analysis revealed two major clusters contained all *O. ficus-indica* (OFI) populations, in addition to cvs. Rossa, Gialla, and Bianca. Regarding the species *O. dillenii*, *O. elata* and *O. robusta* were separately in different branches (Fig. 8.1). The graph has been adapted from the study of Reis et al. (2018)

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# Chapter 9

## Potential Attribute of Crassulacean Acid Metabolism of *Opuntia* spp. Production in Water-Limited Conditions



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and Khaled Y. Kamal

**Abstract** *Opuntia* spp. is an economically important crop from the *Cactaceae* family and native to the tropical and subtropical regions of America. It is cultivated for various uses such as fresh fruit, pharmaceutical, cosmetic, or in the form of beverage. *Opuntia* spp. is well known for its anatomical and physiological characters to conserve water and tolerate extreme drought conditions. The production of *Opuntia* has been extended around the world and it has become an alternative crop in the areas with low-quality soil and water deficit. The ability to tolerate the extreme environments of *Opuntia* species makes them also valuable to study molecular mechanisms underlying abiotic stress tolerance. *Opuntia* is crassulacean acid metabolism (CAM) species with high water-use efficiencies (WUE) that makes it to be able to grow in areas with insufficient precipitation and less water availability to support traditional C<sub>3</sub> or C<sub>4</sub> bioenergy crops. Studies of photosynthesis have shown that these species use a particular photosynthetic pathway known as CAM for the fixation of atmospheric CO<sub>2</sub> during the dry season. Indeed, in the CAM pathway as an adaptation mechanism in plants to water deficit, all or part of the atmospheric CO<sub>2</sub> uptake occurs at the nighttime that remains stomata closer at daytime when the temperature is a higher and consequent reduction in the evapotranspiration rates. Atmospheric CO<sub>2</sub> uptake ratio can affect the biomass production in plants. Despite, CO<sub>2</sub> fixation in CAM plants is 15% more efficient than C<sub>3</sub> plants, CAM species

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often grow slowly due to their extra enzymatic reactions for carbon fixation. In  $C_3$  plants, 3 ATP and 2 NADPH require for each  $CO_2$  molecular fixation, while in CAM plants for each  $CO_2$  fixation, 5.5–6.5 ATP and 2 NADPH are required. In CAM plants net carbon fixation efficiency is 10% less than  $C_4$  plants. However, under optimal growth conditions, *Opuntia* has the potential to produce higher biomass rivaling that of  $C_3$  and  $C_4$  crops. Moreover, growth character and CAM pathway in genera *Opuntia*, allow these species to operate at near-maximum productivity with relatively low water requirement. Cultivation of CAM species from the genera *Opuntia* can provide an alternative to product feedstocks and food in the arid and semiarid regions. For this purpose, here in this chapter, we will discuss (a) the crassulacean acid metabolism pathway; (b) the molecular mechanism of CAM; (c) physiological attributes of CAM during the drought tolerance period; (d) CAM improve *Opuntia* biomass production. Furthermore, screening *Opuntia* species that qualify with water use efficiency and high productivity will be interesting to be reviewed in this chapter to provide the alternative feedstock bioenergy crops that preserve and expand feedstock production in water-limited areas.

**Keywords** Water use efficiency · Molecular mechanism · Drought · Biomass · Arid and semi-arid region

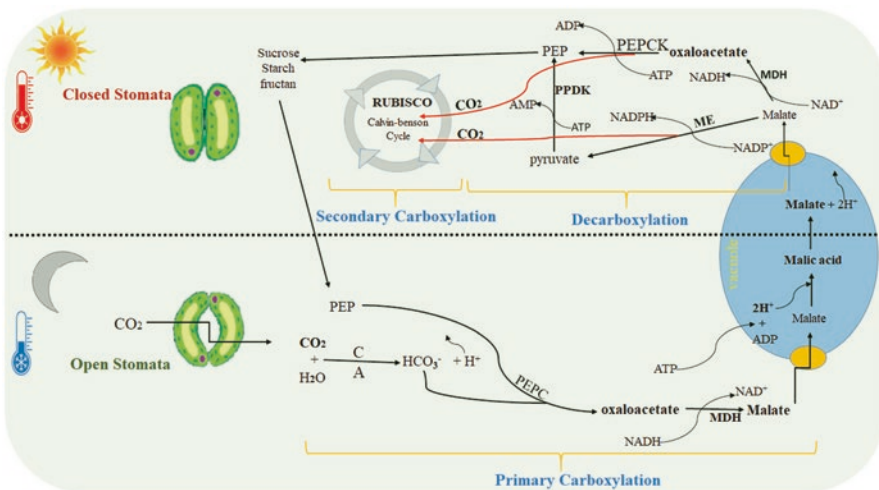
## Abbreviations

ABA	Abscisic acid
CAM	Crassulacean acid metabolism
CAs	Carbonic anhydrases
MDH	Malate dehydrogenase
PEP	Phosphoenolpyruvate
PEPC	Phosphoenolpyruvate carboxylase
PEPCK	PEP carboxykinase
PPDK	Pyruvate orthophosphate dikinase
TF	Transcription factors

## 1 CAM Pathway

Crassulacean acid metabolism (CAM) is common in plants that live in hot and dry environments with limited water. Indeed, the CAM is a photosynthetic adaptation mechanism to water deficit that allows plants to minimize photorespiration by performing the gas-exchange during the night. CAM pathway is distinguished from  $C_3$  and  $C_4$  photosynthesis by the separation of initial  $CO_2$  fixation and Calvin cycle between night and day by opening the stomata at nighttime when the temperature is

cooler (Kondo et al., 2000; Winter & Holtum, 2014). Here we simplified the CAM pathway. In the primary carboxylation that starts by fixing the CO<sub>2</sub> molecules combine with hydroxyl ions (OH<sup>-</sup>) to become HCO<sub>3</sub><sup>-</sup>, which is catalyzed by carbonic anhydrases (CAs) enzyme in the nighttime. In the following reaction, CO<sub>2</sub> molecules are added to a molecule called phosphoenolpyruvate (PEP) by HCO<sub>3</sub><sup>-</sup>, which is catalyzed by phosphoenolpyruvate carboxylase (PEPC) to become oxaloacetate. Oxaloacetate then receives an electron from NADH and becomes a molecule of C<sub>4</sub> organic acid (malate) catalyzed by malate dehydrogenase (MDH) enzyme and accumulate in vacuoles within the plant cells, until the sun rises and photosynthesis begins. During the day, accumulated C<sub>4</sub> organic acids, canonically malic acid but also citric acid is released from the vacuole and decarboxylated by NAD(P)-malic enzyme to provide CO<sub>2</sub> for fixation in the Calvin-Benson cycle behind the closed stomata. Decarboxylation of C<sub>4</sub> acids releases CO<sub>2</sub> and generates pyruvate. The orthophosphate dikinase (PPDK) enzyme catalyzes a reaction to regenerate the pyruvate to the PEP. Depending on the species the decarboxylation can regenerate the oxaloacetate that is catalyzed by NAD(P)-malate dehydrogenase (MDH) and in further reaction, PEP carboxykinase (PEPCK) release the CO<sub>2</sub> and regenerate PEP. The released CO<sub>2</sub> is then refixed by ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) for carbohydrate production *via* the Calvin-Benson cycle (Fig. 9.1) (Borland & Dodd, 2002; Cockburn, 1985; Osmond, 1978; Silvera et al., 2010; Winter, 1985).



**Fig. 9.1** Crassulacean acid metabolism (CAM) pathway. During the night, CO<sub>2</sub> is taken up through the open stomata. In the primary carboxylation reactions, CO<sub>2</sub> is fixed as HCO<sub>3</sub><sup>-</sup> and added to the PEP then converted into the malate by phosphoenolpyruvate carboxylase (PEPC) and store in the vacuole. During the day, behind the closed stomata a decarboxylation reaction occurs to release the CO<sub>2</sub> and regenerate the pyruvate and PEP. The released CO<sub>2</sub> is used by ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) in the Calvin-Benson cycle as a secondary carboxylation phase

Several distinguished CAM modes have been classified depending on developmental and/or environmental influences such as the variety of CO<sub>2</sub> assimilation, stomatal behavior, and acid flux in different plants and plant organs with and without stomata, and between terrestrial and aquatic plants (Cockburn, 1985; Cushman, 2001). In the CAM idling, as very strong CAM, stomata are closed during the day and night time and the day/night organic acid cycle is fed by internal recycling of nocturnally refixed respiratory CO<sub>2</sub>.

This type of CAM generally occurs in severely stressed plants (Griffiths et al., 1989). On the other hand, a weak CAM phenomenon that called CAM cycling occurs in the well-watered plants, where stomata remain closed during the dark period and opened during the light time. In CAM cycling CO<sub>2</sub> fixation occurs in the daytime but at the same time, some nocturnal synthesis of organic acid fed by respiratory CO<sub>2</sub> also occurs. The daytime CO<sub>2</sub> fixation, consequences a direct Calvin-cycle (C<sub>3</sub>-photosynthesis) in addition to the assimilation of CO<sub>2</sub> remobilized from a nocturnally stored organic acid (Sipes & Ting, 1985). Few studies (Guralnick & Jackson, 2001; Lüttge, 2004) have suggested that CAM cycling might be the starter point of CAM evolution in plants. In the obligate CAM species, net CO<sub>2</sub> uptake (up to 99%) occurs exclusively in phase I at nighttime with high nocturnal acid accumulation even under well-watered conditions. However, some net CO<sub>2</sub> assimilation also occurs during the light time in the phases II and IV (Cushman, 2001; Nobel, 1999). Another mode of CAM is facultative or inducible CAM, also known as C<sub>3</sub>-CAM, with a C<sub>3</sub> form of CO<sub>2</sub> fixation and nil organic acid in the normal condition but in the induced state, a small nocturnal CO<sub>2</sub> fixation and organic acid-producing (Smith & Winter, 1996). Facultative CAM species use the C<sub>3</sub> pathway to maximize growth when water availability is sufficient, and changing the C<sub>3</sub> to CAM when they are induced by drought stress in dray seasons (Winter, 1985). High versatility of CAM and CAM-like behavior and the fact that there are no CAM-typical enzymes, make the definition of CAM not straightforward (Holtum, 2002; Lüttge, 2004).

CAM and C<sub>4</sub> pathways are similar in the case of the complement of enzymes and formation of C<sub>4</sub> molecule that later converts into malic acid. Despite these similarities, in C<sub>4</sub> plants, CO<sub>2</sub> assimilation and all of the photosynthetic processes occur during the light, unlike the CAM pathway. CAM is one-cell photosynthesis that does not require the different cell types of mesophyll and bundle-sheath. In the C<sub>4</sub> pathway, the primary carboxylation by PEPC occurs in thin-walled mesophyll cells, and the intermediate organic acids are transferred *via* plasmodesmata to bundle-sheath cells where CO<sub>2</sub> is released and the refixation of the CO<sub>2</sub> by Rubisco takes place (Akhani et al., 2003; Winter et al., 2019). However, some studies reported the coexistence of C<sub>4</sub> and CAM in leaves of C<sub>4</sub> *Portulaca* species (Winter & Holtum, 2014, 2017). In each case of the coexistence of CAM and C<sub>4</sub> pathway, the CAM expression was facultative and not detected in well-watered plants. The location of coexistent C<sub>4</sub> and CAM pathways is not yet detected whether they occur in different regions of the leaf or there is cell sharing (Winter et al., 2019). CAM plants are taxonomically five times more numerous than C<sub>4</sub> plants. However, from the 150 most intensively cultivated plants, about 10 use the C<sub>4</sub> pathway, and only 2 use the CAM pathway (Nobel, 1991; Winter et al., 1983). Despite, the higher CO<sub>2</sub> fixation and

water use efficiency, CAM plants grow relatively slowly compare to the C<sub>3</sub> and C<sub>4</sub> plants (Black, 1973; Winter, 1985). The extra enzymatic CAM pathway requires 5.5 or 6.5 ATP and 2 NADPH that is equal to 715–765 kJ mol<sup>-1</sup> per CO<sub>2</sub> molecules to incorporate into photosynthetic products, whereas the C<sub>3</sub> pathway requires 3 ATP and 2 NADPH, and C<sub>4</sub> pathways require 4 or 5 ATP and 2 NADPH. Thus, CAM plants are 20% less efficient than C<sub>3</sub> plants and 10% less efficient than C<sub>4</sub> plants in terms of energetic costs for CO<sub>2</sub> fixation (Edwards & Walker, 1983; Salisbury & Ross, 1992). Despite the less energy requirement of the C<sub>3</sub> pathway, CAM plants have 15% more net CO<sub>2</sub> fixation than C<sub>3</sub> plants due to the photorespiratory cycle in the C<sub>3</sub> pathway that releases about 0.5 CO<sub>2</sub> per O<sub>2</sub> in oxygenase activity of RuBisCo. However, the net CO<sub>2</sub> fixation in CAM plants might be 10% less efficient than C<sub>4</sub> plants (Taiz & Zeiger, 1991).

Many endemic plants from the arid and semiarid regions with temporary water availability use C<sub>4</sub> or CAM photosynthesis pathways that improve WUE compare to the C<sub>3</sub> photosynthesis of most plants. C<sub>4</sub> plants such as corn and sugar cane are typically summer plants that can sustain high heat and reduced water supply to some extent. On the other hand, CAM plants like cacti are better suited for arid environments with the ability to store water in their organs.

## 2 Molecular Mechanism of CAM

CAM photosynthesis is an evolutionary photosynthetic metabolism adaptation in plants to heat and drought that can improve WUE and crop production under water-deficit conditions (Borland et al., 2014; Heyduk et al., 2019). Various strategies have been proposed to improve agricultural productivity by increasing photosynthesis (Evans, 2013; Silvera et al., 2010). Understanding the genetic basis of CAM is essential for the potential moving of CAM into C<sub>3</sub> plants to maximize WUE. Facultative CAM mode has been used as a model to identify the CAM pathway functional gene set and understanding the regulation of these genes (Hartwell et al., 2016). The first attempt to present a comprehensive overview of the genes regulating the CAM pathway was performed (Cushman & Bohnert, 1997). The key enzymes in CAM and C<sub>4</sub> pathways such as PEPC, PPK, NAD(P)-ME, and PEPCK are present in all higher plants and no unique metabolic pathways are required in CAM plants (Cushman & Bohnert, 1997; Gowik & Westhoff, 2011). CAM-specific PEPC isoforms might be evolved for the first time in response to water deficit from ancestral non-photosynthetic isoforms by gene duplication (Gehrig et al., 2001). It thought that the CAM pathway has evolved independently multiple times from ancestral C<sub>3</sub> plants (Silvera et al., 2010).

Plasticity in the expression of CAM is the central mechanism to optimize the carbon fixation in CAM plants. The expression level of CAM enzymes change temporally in CAM plants in response to the environmental factors, such as drought, salinity, and light because of the alterations in their regulation, and particularly overexpressing compare to C<sub>3</sub> plants (Cushman et al., 2008; Cushman & Bohnert,

1997; Heyduk et al., 2018; Lepiniec et al., 1993; Yin et al., 2018). PEPC activity is thought to be a major factor in limiting the magnitude of CAM. Therefore, the mechanisms that regulate PEPC activity at nighttime, could play a key role in CAM expression (Taybi et al., 2004). During the CAM cycle, phosphoenolpyruvate carboxylase Kinase (PEPC Kinase) enzymes sets up the day/night regulation of PEPC by catalyzing the phosphorylation of PEPC at nighttime (Nimmo et al., 1986). Taybi et al. (1995) reported that the transcript abundance of PEPC genes (*Ppc*) in two CAM-performing species of *Clusia* genus was higher than the other two  $C_3$  species and prolonged drought treatment had no detectable effect on the levels of *Ppc* transcripts in these species. Thus, transcriptional control of PEPC abundance might be a key factor for the genotypic capacity of CAM in the *Clusia* genus (Taybi et al., 2004). Similar results showed that an increase in the PEPC expression/amount had an important effect on photosynthetic diversification on  $C_4$  photosynthesis within the genus *Flaveria* (Engelmann et al., 2003). Despite, the changes in the expression level of *Ppc* genes in response to the drought in two  $C_3$  species of the genus *Clusia* the transcript levels of PEPC genes (*Ppck*) were relatively constant over the 24-h cycle comparable in both species displayed with no CAM activity (Taybi et al., 2004). PEPC phosphorylates PEPC during the dark period that causes a reduction in PEPC sensitivity to feedback inhibition by malate. Interestingly, the cytosolic malate concentration and transportation into and out of the vacuole processes play a crucial role in regulating the *Ppck* gene expression in CAM plants (Borland et al., 1999; Nimmo, 2000). Silencing of PPCK in *Kalanchoë fedtschenkoi* reduced the CAM activity by ~66% and also perturbs central components of the circadian clock, suggesting PPCK may be essential for clock robustness (Boxall et al., 2017).

In the induced CAM, abscisic acid (ABA) plays an important role as an induction signal (Chu et al., 1990). ABA receptors modulate the activity of their downstream targets, ranging from kinases to anion channels and numerous transcription factors (TFs). ABA receptors interact with clade A PP2C phosphatases (PP2CA) to prevent the dephosphorylation of SNF1-RELATED PROTEIN KINASES 2 (SnRK2). Autophosphorylated SnRK2s subsequently modulate the activity of their downstream targets, which recognize ABA-responsive elements in the promoter sequences of target genes. A recent study in *Talinum triangulare* as a model plant for induced CAM indicated that the central circadian clock was not affected by ABA treatment and most changes in the gene expression level were observed after the dark period. However, ABA treatment altered the transcript levels of genes encoding carbonic anhydrases (CAs). These results suggesting a combined effect of ABA and darkness on transcript abundances in induced CAM pathway.

The impact of transcription factors (TFs) on the regulation of the genes in response to stress have been reported previously (Cai et al., 2014; Chen et al., 2014; Swain et al., 2017; Yang et al., 2012). A set of candidate CAM TFs involved in  $C_3$  to CAM transition has been reported in obligate CAM species of *Agave*, *Kanlanchoe* and *Manfreda* (Heyduk et al., 2018; Huang et al., 2018; Moseley et al., 2018; Yin et al., 2018). Moreover, in weak CAM species of *Polianthes* and *Beschorneria* (Heyduk et al., 2018) and facultative CAM in *Tralinum triangulare* (Brilhaus et al., 2016), several CAM pathway-related TFs have been identified. Overexpression of MYB59 (myeloblastosis closest ortholog in Arabidopsis), the candidate CAM TF



from *K. fedtschenkoi*, increased integrated WUE, and biomass in the transgenic *A. thaliana* compared to WT plants (Amin et al., 2019). Recently, Maleckova et al. (2019) used *T. triangulare* as a facultative CAM model to study the changes in transcriptome and metabolome during the induction phase in response to the ABA signal. The temporal patterns of numerous TFs acting downstream such as ABA-Binding, ABA-synthesis, and transport proteins influenced by ABA treatment and remained increased. The expression modulation of CAM abiotic stress-responsive gene by the appropriate CAM TFs generated stress-adaptive phenotypes in *A. thaliana* and likely other  $C_3$  plants. These results shows that the CAM abiotic stress-responsive genes are also present and conserved in  $C_3$  plants (Amin et al., 2019; Heyduk et al., 2018).

A recent study indicated that long non-coding RNAs (lncRNAs) extracted from pineapple leaves show diurnal expression patterns, similar to the clock-regulated genes. Few of these lncRNAs that showed competing for endogenous RNAs (ceRNAs) activities, were identified for PEPC and PPCK genes demonstrate that leaf lncRNAs play an important role in CAM photosynthesis in pineapple (Bai et al., 2019). CeRNAs protect the genes from repression by binding to and sequestering specific miRNAs (Yuan et al., 2017). Two microRNAs, miR164c-3p and miR166e-3p were identified in the pineapple with potential regulatory effects in PPCK1 and MDH genes, respectively (Wai et al., 2017). Moreover, gene co-expression network analysis from the temporal RNA-seq data in pineapple leaves, indicate that two clusters enriched of CAM-related genes, including CA, PEPC, PPCK, NAD-ME, MDH, and PPDK with different biological functions. One of these clusters, enriched with a significant number of core circadian clock genes, including CCA1/LHY, GIGANTEA, PSEUDO-RESPONSES REGULATOR 7, and PSEUDOREPRESONSES REGULATOR 9 with circadian-related cis-acting elements including the G-box (CACGTG), and evening motifs (AAAATATCT), and CCA1 (AAAAATCT) binding sites (Wai et al., 2017). This result supports the hypothesis that CAM activity could be engineered through the reprogramming of regulatory regions of pre-existing  $C_3$  genes that cues stomatal opening and  $CO_2$  uptake in the nighttime.

### 3 Physiological Attributes of CAM During the Drought Tolerance Period

In many tropical and subtropical areas, CAM plants are the dominant vegetation because of their ability to withstand multiple, synergistic stressors in harsh environments (Lüttge, 2004). CAM plants present a reverse stomatal conductance pattern by uptaking  $CO_2$  at night when the temperature is low. Therefore, stomata movements are essential in controlling of transpiration rate and increasing the WUE (Grondin et al., 2015; Maleckova et al., 2019; Ming et al., 2016). A great number of stomatal movement-related genes are reported in *Ananas comosus*, *Phalaenopsis equestris*, *Arabidopsis*, rice, and sorghum (Chen et al., 2020). The *Cis* elementary site analysis of those genes indicate that the CIRCADIAN CLOCK ASSOCIATED

1 (CCA1)-binding site (CBS; AAAAATCT) and G-box binding site (CACGTG) enriched 10% or higher frequency than the expected frequencies based on random chance in *A. comosus* var. *comosus* as a CAM species. Moreover, motif ERF73, ERF7, and ABR1 were enriched in *A. comosus* var. *comosus* and var. *bracteatus* (CAM species) compare to rice, sorghum, and *Arabidopsis* as C<sub>4</sub> and C<sub>3</sub> plants (Chen et al., 2020).

It has been shown that ABA plays a major role in stomatal closure in CAM plants (Taybi et al., 1995). ABA is synthesized under drought stress conditions at the leaf and causes activation of two types of plasma membrane anion channels, called slow-sustained (S-type) and rapid-transient (R-type) anion channels in guard cells. It would lead to the depolarization of guard cell membranes triggering osmotic ion (K<sup>+</sup>) efflux and the loss of guard cell turgor resulting in stomatal closure (Geiger et al., 2009; Munemasa et al., 2015). Although it has been known that ABA can operate in guard cells via Ca<sup>2+</sup>-dependent signal transduction pathways (Leckie et al., 1998). ABA activates the plasma membrane Ca<sup>2+</sup>-permeable cation (I<sub>Ca</sub>) channel resulting in guard cell cytosolic free Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>cyt</sub>) elevations and Ca<sup>2+</sup> release from intracellular Ca<sup>2+</sup> stores (Laanemets et al., 2013). The molecular mechanism of ABA regulation of Ca<sup>2+</sup> releasing from the intracellular Ca<sup>2+</sup> stores remains unknown (Munemasa et al., 2015). Besides the daytime closure of stomata that reduces the loss of water, the osmotic effect of nocturnal accumulations of vacuolar organic acids allows nocturnal acquisition of water from the transpiration stream and also from dew, and transitory storage of water in vacuoles (Lüttge, 2004).

The circadian rhythms of CO<sub>2</sub> fixation in CAM plants occur in response to the darkness and temperature due to the periodic activity of the primary carboxylase (PPC) and secondary carboxylase (Rubisco) (Wilkins, 1992). Both primary and secondary CO<sub>2</sub> fixation is localized within individual leaf mesophyll cells. The CAM pathway requires strict temporal regulation of enzyme activities, membrane transporters, and their regulatory proteins (Wilkins, 1992; Wyka et al., 2004). Moreover, the correlation between the leaf anatomy and CAM pathway has been reported in *Clusia*, *Annanas*, *Kalanchoe*, and *Yucca* (Heyduk et al., 2016; Nelson & Sage, 2008). In *Clusia* genus, CAM species have thicker and more succulent leaves with lower internal air space than C<sub>3</sub> species (Barrera Zambrano et al., 2014). *Yucca gloriosa*, a hybrid of *Yucca aloifolia* (CAM species) and *Yucca filamentosa* (C<sub>3</sub> species) exhibit intermediate C<sub>3</sub>-CAM with intermediate leaf thickness, succulence, and internal air space. It seems that these traits represent fundamental requirements for the CAM cycle. It has been hypothesized that an increase in succulence, and the corresponding decrease in internal air space, limiting the transition of gases through the mesophyll which helps CAM plants to keep CO<sub>2</sub> inside the leaf during decarboxylation in the daytime (Heyduk et al., 2016). However, CO<sub>2</sub> diffusion is not always related to the leaf thickness or succulence in CAM species. *Agave tequilana* has higher recorded values of instantaneous and integrated net CO<sub>2</sub> uptake compare to *Kalanchoe daigremontiana* that seems to be related to higher stomatal density and more aerated chlorenchyma in *A. tequilana* (Owen & Griffiths, 2013).

CAM pathway is highly plastic and the expression of CAM is intimately linked to the range of environmental conditions including CO<sub>2</sub>, water, light, and

temperature (Borland et al., 1999; Lüttge, 2004; Winter et al., 2008). The plasticity of the CAM pathway reflects the multiple origins of CAM and its utility in different environments (Cushman, 2001). The plasticity of CAM can provide a selective advantage in the regions with seasonal changes in water availability and light intensity (Borland et al., 1992; Winter et al., 2009). In some of the CAM species, the activity of a specific PEPC increases during the spring and summer and decline during autumn and winter (Pilon-Smits et al., 1991). It has been reported that net CO<sub>2</sub> uptake depresses significantly at the end of the day in darker seasons in *Aechmea* 'Maya' plants (Ceusters et al., 2009). In the facultative and CAM cycling species, a flexible photosynthetic system occurs switching from C<sub>3</sub> to CAM under water deficit conditions (Wai et al., 2019). The activation and deactivation of PEPC during the night (Phase I) and day (Phase III) plays the major key in circadian CO<sub>2</sub> uptake in CAM plants. The deactivation of PEPC at the start of the day (Phase II) and reactivation at the end of the day (Phase IV) may prolong, depending on species and environmental factors (Borland & Griffiths, 1997). Under changing environmental conditions, circadian control of PEPC activation plays a crucial role in the CAM pathway flexibility for optimizing carbon gain (Borland et al., 1999). The day/night turnover of carbohydrate and Rubisco/PEPC reciprocally activation status in different phases are also determining the magnitude of C<sub>4</sub> carboxylation (Borland & Dodd, 2002; Maxwell et al., 2002).

Light intensity (photosynthetic photon flux density, PPFD) is a critical environmental factor for determining the magnitude and duration of each of the four phases of CAM. The rate of organic acid mobilization from the vacuole is determined by PPFD during Phase III which might be linked to the Rubisco mediated net CO<sub>2</sub> uptake (Lüttge, 2004; Thomas et al., 1987). PPFD also determine the abundance of carbohydrate generated *via* the Calvin cycle and gluconeogenesis which is subsequently required for the nocturnal provision of PEP (Nobel & Hartsock, 1983). The light quality also can affect the CAM pathway that implies important roles for certain photoreceptors in synchronizing metabolism over the diel phases (Ceusters et al., 2014). The environmental depending CAM plasticity especially the seasonally related CAM plasticity might affect carbohydrate partitioning and biomass enhancement of CAM plants.

## 4 CAM Improve *Opuntia* Biomass Production

*Opuntia* spp. is widely distributed in the arid and semi-arid regions with economic potential in agriculture because of their great anatomical and physiological adaptations to the long periods of drought and high temperature (Edvan et al., 2020; Nobel & De la Barrera, 2003). *Opuntia* species have been used as food resources and traditional medicine for their nutritional properties, also as a forage crop in the areas with limited rainfall (Díaz et al., 2017; Nunes et al., 2017). Despite the wild distribution of *Opuntia* species, different degrees of domestication have been observed in some of the species (Reyes-Agüero & Rivera, 2011). Nowadays, *Opuntia* plants are

grown in more than 30 countries as the most important agricultural cactus crop wherein Mexico and Italy are the main producer countries, respectively (Díaz et al., 2017).

Several adaptation mechanisms including the shallow root system and thick, waxy cuticle aid *Opuntia* species to tolerate the drought by rapid water uptake and preventing excessive water loss (Ogburn & Edwards, 2010). In addition to the morphological properties, the CAM photosynthesis pathway, that allows plants to uptake atmospheric CO<sub>2</sub> at night when water loss is minimized, is the most physiological adaptation of *Opuntia* species to the drought and high temperature (Cui et al., 1993). Indeed, the CAM pathway is present in more than 6% of vascular plant species as an adaptation mechanism to water deficit (Cushman, 2001). CAM plants show remarkable metabolic plasticity for modulating nocturnal and diurnal CO<sub>2</sub> uptake (Borland et al., 2009). In the constitutive CAM species such as *Opuntia basilaris*, *Opuntia ficus-indica*, and *Opuntia stricta*, the CAM cycle is constitutively expressed in plants (Nobel, 1985). However, young plants and young photosynthetic tissue of constitutive CAM species may exhibit C<sub>3</sub> photosynthesis with daytime CO<sub>2</sub> uptake, which decreases the plants and tissues maturation (Winter & Holtum, 2002). *Opuntia elatior* is an example that exhibits strong C<sub>3</sub> and facultative CAM photosynthesis in young plants in contrast with obligate CAM in the mature plants. C<sub>3</sub> photosynthesis, drought-stress-related facultative CAM in the young plants, and developmentally controlled constitutive CAM can all contribute to the early growth of *O. elatior* (Winter et al., 2011). Despite, CAM is the most important physiological adaptation mechanism to cope with drought stress in plants, the plasticity of CAM and some other morphological and physiological traits can affect the tolerance rate of the *Opuntia* species. For instance, cultivated *O. ficus-indica* does not stop forming the daughter cladodes even under severe drought conditions. The fact that daughter cladodes exhibit C<sub>3</sub> photosynthesis with the daytime stomatal opening in the early developmental stage, cause water loss, while, the wild type stop the development of the daughter cladodes in response to the drought stress that, allows them to avoid the unfavorable condition (Pimienta-Barrios et al., 2005). In obligate CAM plants, which are native to arid regions, frequently severe drought stress cause to lose phase IV and reduction of phase II (Nobel, 1985). In *O. ficus-indica* during phase II, both CO<sub>2</sub> uptake and stomatal aperture are maintained in both mother and daughter cladodes, even during prolonged drought. However, phase IV is curtailed (Pimienta-Barrios et al., 2005). The fact that phase IV occurs in the late afternoon when the temperature is still high, the terminated phase IV is essential to tolerate the drought in CAM plants (Dodd et al., 2002). In the obligate CAM plants such as some *Opuntia* species, all CAM phases are expressed when water availability is sufficient. When drought stress starts, first, Phase IV becomes reduced and then suppressed while Phase II becomes limited. In the more severe drought situations, Phase I declines, and stomata also begin to close in the dark period. When closed stomata do not allow CO<sub>2</sub> acquisition from the atmosphere, photosynthesis can still run using respiratory CO<sub>2</sub> recycled from nocturnal respiration via PEPC and vacuolar organic acids (Lüttge, 2004). This ability of CAM plants allows them to continue photosynthesis and biomass production even under

severe drought. However, when the CAM plants are exposed to both drought and cold stress, negative carbon balance occurs because of the closed stomata even during the nighttime (Koch & Kennedy, 1980). Moreover, developmental factors may also be involved in the duration and/or magnitude of CAM activity in *Opuntia*. Maximum CAM activity has been found during the shoot initiation and especially, in the flowering time (Brulfert et al., 1975). The plasticity of CAM is highly under the effects of some environmental factors. The elevation of atmospheric CO<sub>2</sub> affects the CAM cycle and biomass productivity in *Opuntia* species (Cui et al., 1993). *Opuntia ficus-indica* is an extremely productive species with a C<sub>3</sub>-CAM pathway, which response to elevated CO<sub>2</sub> by increasing the daytime CO<sub>2</sub> uptake using the C<sub>3</sub> pathway, resulting in increases in the biomass accumulation in *O. ficus-indica* (Nobel & Israel, 1994). The daytime CO<sub>2</sub> uptake can be affected by other environmental factors especially temperature and water availability. Despite, the young cladodes of *O. ficus-indica* show the typical patterns of CAM, their low water storage ability is not sufficient to cope with the drought stress (Pimienta-Barrios et al., 2012). Succulence have been considered one of the most efficient adaptations for maintaining carbon gain in CAM plants that allows plant to remain active during the seasonal water shortages (Ogburn & Edwards, 2010).

*Opuntia* species such as *Opuntia elatior* not necessarily exhibit CAM-photosynthesis during seedling stages when water from rainfall is plentiful. The expression of C<sub>3</sub> photosynthesis in *O. elatior* after germination provides relatively higher growth rates and assists plant establishment when water is still available (Winter et al., 2011). In Cactaceae family members such as *Opuntia streptacantha* seedlings establish mostly under the canopy of nurse plants (Yeaton & Manzanares, 1986). Shade and higher soil humidity under nurse plants might protect the young seedling from the high temperature but, it has been reported that in some cactus seedlings such as *Ferocactus acanthodes*, the photosynthesis, and growth of the seedling under the canopy of nurse plants with diminished light were lower than under direct sunlight (Franco & Nobel, 1989). It has been shown that *O. streptacantha* seedlings can osmotically adjust to tolerate drought, similar to adult *Opuntia* plants. Moreover, the relative growth rate of the *O. streptacantha* seedlings not decreases under water deficit condition in contrast with other cacti species, probably because *O. streptacantha* is an obligatory CAM species (Delgado-Sánchez et al., 2013). In the shaded cactus seedling, the nocturnal acid accumulation increases but decreases for the light-exposed cactus seedlings, regardless of their water treatment (Gallardo-Vásquez & De la Barrera, 2007). Thus, the presence of CAM in shaded cactus seedlings can increase WUE and reduce the risk of photoinhibition. Nevertheless, *O. streptacantha* seedlings grow well in the well-watered conditions, but this condition is not so common in arid and semi-arid environments. Despite the irrigation plays an important role in CAM expression in obligate CAM species like *O. basilaris*, even under continual irrigation conditions CO<sub>2</sub> uptake doesn't change from CAM to the daytime CO<sub>2</sub> fixation. However, the prolonged drought and high temperature can magnify the CAM and convert to an idling mode in which organic acids fluctuate, but there is no exogenous CO<sub>2</sub> uptake. Presumably, the persistence of acid metabolism maintains the cacti in a near positive energy balance allowing

persistence for extended unfavorable periods (Hanscom & Ting, 1978). Among the *Opuntia* species, *Opuntia ellisiana* has one of the greatest WUE among the CAM, C<sub>3</sub> and C<sub>4</sub> plants. Despite, this species is slowly growing and not useful species, but it is completely cold hardy and can store a significant quantity of water that could be of use for animal drinking water (Han & Felker, 1997). It has been widely accepted that, in general, plants possessing CAM have higher WUE than C<sub>3</sub> and C<sub>4</sub> plants, which makes them a great adapted species to the arid and semi-arid regions.

Approximately 50% of the global land area is considered arid, semi-arid, or dry sub-humid regions (Zika & Erb, 2009). Moreover, the rising global temperatures, declining water availability, and rising CO<sub>2</sub> concentrations all emphasize the importance of using the drought-tolerant crops with higher WUE. *Agave* (Agavaceae) and *Opuntia* species are considered low-input perennial crops that are operated at near-maximum productivity with relatively low water requirements (Cushman et al., 2015). The genus *Opuntia* contains ≈180 species and prickly pear cactus (*Opuntia ficus-indica* Mill.) is the most commonly cultivated species, because of multipurpose usage, as human food, animal fodder, in medical applications, for wastewater treatment, as a host plant for cochineal insects in carmine-red production, as live hedges, erosion control, and land reclamation and rehabilitation (de Cortázar & Nobel, 1992; Paiva et al., 2016). Despite, the *Opuntia* species grow slowly, compared to C<sub>3</sub> and C<sub>4</sub> species, typically display higher shoot to root ratios (up to 10:1). When crop water demands for some *Opuntia* species average only 16% of those for C<sub>3</sub> crops, and 28% for C<sub>4</sub> crops, above-ground biomass productivities are comparable or may even exceed those reported for C<sub>3</sub> and C<sub>4</sub> species (Borland et al., 2009). Low productivities associated with CAM plants are normally the result of seasonally arid conditions (de Cortázar & Nobel, 1992). However, some important factors such as low plant density and low soil fertility levels also reducing the output of *Opuntia* species (Dubeux et al., 2006).

The irrigation, high soil fertility, and the interception of nearly all of the incident photosynthetic photon flux (PPF) by photosynthetically active tissue are the important factors contributing to the high productivity in *Opuntia* species. Despite the importance of *Opuntia* species in modern agriculture, different aspects of their ability to tolerate the unfavorable environmental conditions are still unknown. Moreover, different genotypes of cultivated *Opuntia* species can exhibit different productive traits in responses to the different locations of cultivation (Edvan et al., 2020). Further studies are required for a better understanding of the molecular and physiological mechanisms of their stress tolerance abilities to assess the potential of each genotype for specific locations.

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# Chapter 10

## Harvest and Postharvest Technology of *Opuntia* spp.



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**Abstract** *Opuntia* spp. are among the most important potential crops in the sustainable agricultural development of arid and semi-arid regions. They are mostly known as a fruit crop, and it is slowly achieving the status of the formal crop; however, there is an increased interest in *Opuntia* fruit and cladode production due to the great demand for human and animal nutrition in the global market. Fruit are harvested based on peel color, fruit size, fullness and flattening of the floral cavity or receptacle. They are non-climacteric and highly perishable with a short shelf life of few days under marketing conditions. Shelf life is mainly affected by decay, which is related to physical damages during harvest and handling. The fruit is also susceptible to chilling injury when exposed to a prolonged cold temperature below 9 °C. For harvested cladodes, the acid content and flavor may fluctuate significantly during the day and can also be affected by postharvest storage temperature. Therefore, to reduce decay, maintain quality, and prolong the shelf life of *Opuntia* fruit and cladode, this chapter will discuss on harvest methods, as well as postharvest physiology and technology of *Opuntia* species that are used for the production

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of fruit, as well as cladodes that are widely used as a type of vegetables for human nutrition, in addition to those used as forage for animal feed.

**Keywords** Cactus pear · Prickly pear · Harvest · Storage · Temperature · Fruit quality · Cladode · Forage · Firmness · Decay · Chilling injury · TSS · Ascorbic acid

#### Abbreviations

CA Controlled atmosphere  
CAI Cladode area index  
CAM Crassulacean acid metabolism  
CI Chilling injury  
CP Crude protein  
DM Dry matter  
DW Dry weight  
EVA Ethylene-vinyl acetate  
FC Fresh cut  
IMZ Imazalil  
MAP Modified atmosphere packaging  
OM Organic matter  
PPO Polyphenol oxidase  
PVC Polyvinyl chloride  
RH Relative humidity  
SOPP Sodium orthophenilphenate  
TBZ Thiabendazole  
TSS Total soluble solids  
WL Weight loss

## 1 Introduction

The family Cactaceae has substantial agricultural and economic importance due to their uses as ornamental plants, as well as it has an essential role in the production of nutritional, medicinal, and forage components (Feugang et al., 2006, Ortega-Baes et al., 2010; Kumar et al., 2018). In this regard, the subfamily Opuntioideae includes *Opuntia*, known as prickly pear or cactus pear, which includes several species that produce nutritious fruit and edible cladodes, a type of fruit and vegetable, respectively (Saenz, 2013). The evolution of *Opuntia* in arid and semi-arid environments, known for water scarcity and poor soils, has led to adaptive anatomical, morphological, phenological, and physiological traits (Sudzuki Hills, 1995; Felker et al., 1997). Asynchronous reproduction and Crassulacean Acid Metabolism (CAM), associated with structural adaptation of the stem and leaves to cladodes and

thorns, enable the plant to survive and reach acceptable productivity under such harsh environmental conditions (Pimienta-Barrios, 1994; Cuerrero-Beltran & Ochoa-Velasco, 2018).

Cacti' flowers are yellow or red with a pink cover, and they arise from the top of the cladodes producing green, yellow, red, or purple berry fruit (Anderson, 2001), based on the betalain pigment content (Fernandez-Lopez & Almela, 2001; Sepulveda et al., 2003; Stintzing et al., 2005). Fruit exhibits a double growth curve with a total of 11–12 weeks from pollination. The fruit grows rapidly after set showing an increase in weight, length, and diameter until the age of 64 days, and then the growth rate decreases sharply for 7–10 days. Afterward, fruit resumes growth with a rapid increase in size and weight until fully ripe (Sudzuki Hills, 1995). The cactus pear's fruit is palatable due to the high sugar and low acid content (pH = 5.3–7.1) (Sawayat et al., 1983b; Sepulveda & Saenz, 1990). The pericarp of ripe fruit accounts for 33–55%, while the pulp is 45–67%, and the seeds are 2–10% of the total fruit weight (Piga, 2004). Ripe fruit weight varied from 110 to 160 g with about 12–15% sugar content and 0.05–0.18% citric acid (Felker et al., 1997; Wolfram et al., 2002; Abd El-Hameed et al., 2014). The nutritional benefits of fruit and cladodes are mainly related to their antioxidant properties (Piga, 2004; Cota-Sanchez, 2016; Berrabah et al., 2019). The antioxidant potential of the fruit is two times higher than any other fruit species due to the high levels of vitamins B6 and C and nutrients like calcium (Ca), potassium (K), copper (Cu), iron (Fe), magnesium (Mg) and manganese (Mn) (Tesoriere et al., 2005; Albano et al., 2015). Fruit contains higher vitamin C (180–300 mg kg<sup>-1</sup>) than the apple, pear, grape, and banana, while other vitamins (e.g., A, B1, B2, B3, E, and K) are in trace (Lanuzza et al., 2015). Lipids, protein, minerals, and fibers do not differ significantly from other tropical fruit. The large quantity of insoluble fibers present in the seeds provides the major source of fibers (Bensadon et al., 2010). Lipids are present in the peel, pulp, and seeds. Peel, a by-product, gives appreciable amounts of polyunsaturated fatty acids, mainly linoleic acid,  $\alpha$ -tocopherol, sterols,  $\beta$ -carotene, and vitamin K1. Linoleic acid is also found in pulp and seed oil, while pulp oil predominates in  $\gamma$ - and  $\alpha$ -linolenic acids. The fruit also has a high content of free amino acids, particularly proline, glutamine, and taurine (Salim et al., 2009; Yahia & Mondragon-Jacobo, 2011; Slimen et al., 2016; Kumar et al., 2018; Garcia et al., 2020). The fruit is eaten fresh, processed into food, drink or as a source of natural colors, food supplements, cosmetic components (Mobhammer et al., 2006, Yahia & Saenz, 2011; El-Neney et al., 2019), and pharmaceutical ingredients to treat several diseases (Galati et al., 2003a, b; Palumbo et al., 2003; Wolfram et al., 2003; El-Mostafa et al., 2014; Diaz et al., 2017).

Known as nopalitos or nopal, tender cladodes can also be used raw or cooked for human nutrition. They can be renewed by continuous harvest (Sahagún, 1997) at an interval of 15–20 days when they reach 10–15 cm in length and an average weight of 30 g that can provide an average yield of 1.5 kg tender nopal plant<sup>-1</sup> year<sup>-1</sup>. Several potentially active nutrients and their multifunctional properties are found in the nopal. Mainly nopal is consumed fresh, canned, or pickled, as well as for culinary and salad. Therefore, there is a need to improve product shelf life (Kumar et al., 2018). Nopal is rich in pectin, mucilage, phenolics, nicotiflorin, vitamins,

polysaccharides, polyunsaturated fatty acids, amino acids (proline, taurine, serine, glutamine, leucine, lysine, valine, arginine, phenylalanine, and isoleucine) and different nutrients, mainly K and Ca. Nopal also contains antioxidants and various flavonoids, particularly quercetin 3-methyl ether, a highly efficient radical scavenger (Fernandez-Lopez et al., 2010; Chandra et al., 2019). Nopal is characterized by high malic acid content due to CAM-based diurnal rhythm (Osorio-Esquivel et al., 2011; Oumato et al., 2016; Saroj et al., 2017; Yahia & Sanes, 2018).

Cactus, as a succulent and drought-tolerant plant, can produce  $>20 \text{ t ha}^{-1} \text{ year}^{-1}$  of dry matter (DM) and provide  $180 \text{ t ha}^{-1} \text{ year}^{-1}$  of water stored in its cladodes, representing a cost-effective option for livestock nutrition (Dubeux et al., 2015). The importance of cactus pear concerning animal fodder has been increased to improve the used techniques for proper utilization as a livestock feed source. Cactus pear cladode quality does not deteriorate on storage, and it maintains the green color and vitamin A level; hence, it can be an excellent source of fodder during drought periods (Felker et al., 1997). Compared with conventional fodder types, *Opuntia* cladodes have high ash content ( $100\text{--}250 \text{ g kg}^{-1} \text{ DM}$ ) (Sawyer et al., 2001). Cactus cladodes are also high in carbohydrates ( $600 \text{ g kg}^{-1} \text{ DM}$ ), starch ( $75 \text{ g kg}^{-1} \text{ DM}$ ) and  $\beta$ -carotene ( $6.5 \text{ mg kg}^{-1} \text{ DM}$ ) (Ayadi et al., 2009), soluble nitrogen (N) ( $865 \text{ g kg}^{-1} \text{ DM}$ ) (Ben Salem et al., 2002), Ca ( $29\text{--}42 \text{ g kg}^{-1} \text{ DM}$ ) and phosphorus (P) ( $3\text{--}4 \text{ g kg}^{-1} \text{ DM}$ ) (Batista et al., 2003b). Cow consuming 40 kg of fresh cactus per day will also consume 35 L water per day. Thus, cacti are suitable fodder crops for animals living in the arid and semi-arid ecosystem (Kauthale et al., 2017). It is reported that a daily ratio of 40 kg of cactus, 0.5 kg of mineral salts, and 0.5 kg of protein supplement is sufficient to permit excellent live weight gain, reproduction, and lactation from nursing cattle (Kumar et al., 2018).

This chapter will cover the harvest methods and postharvest techniques that satisfy the various uses of *Opuntia* spp., maintaining the nutritional value for human or animal feed and other industrial applications.

## 2 Harvest and Postharvest Technology for Fruit Production

### 2.1 Fruit Yield

*Opuntia* bloom once a year, although it has been reported to bloom twice a year in Italy (Inglese, 1999). Plants start to flower after the third year of the plantation, and fruit can be harvested in 11–12 weeks from flowering. Fruit yield is extraordinarily erratic and vary greatly, not only from one country to another but also within orchards of the same cultivar (Sahagún, 1997). Harvest season starts from April to August in the Americas (Scheinvar, 1995), November to December in the Mediterranean basin (Le Houerou, 1996) and, mid-June to late August in Egypt (El-Saedy & El-Naggar, 2009). Fruit usually does not reach maturity or ripening stage simultaneously within the same orchard; therefore, the harvest window takes



about 30–50 days on average. Fruit yield varies from 1 to 5 t ha<sup>-1</sup> under traditional agricultural practices to 15–30 t ha<sup>-1</sup> with intensive orchard practices under rain-fed conditions of 40–60 cm year<sup>-1</sup> (Monjauze & Le Houerou, 1965). The possible reasons for high variability in fruit yield are genotype characteristics, environmental conditions, orchard design, and cultural practices (Nerd et al., 1991; Inglese, 1995; Inglese et al., 2002; Potgieter, 2007). Increased fruit availability on markets could be achieved by growing cactus pear in diverse agroclimatic areas (Mondragon-Jacobo et al., 2009; Liguori & Inglese, 2015), using cultivars with different ripening periods (Gallegos Vazques et al., 2006), improving postharvest technology (Liguori & Inglese, 2015), and adopting crop manipulation techniques (Barbera et al., 1992a; Brutsch & Scott, 1991).

## 2.2 *Maturity and Harvest Indices*

Quality is a significant factor in fruit production since consumers prefer attractive fruit with a good taste and high nutritional value. The overall quality is generally the highest at harvest (Cota-Sanchez, 2004) and gradually declines at rates that vary according to genetic background, preharvest treatments, environmental conditions, degree of maturity at harvest, handling processes, postharvest treatments, and storage and marketing conditions (Potgieter & D'Aquino, 2017). Overall quality varies based on the targeted consumers, market destination, and storage time. As the fruit matures, their nutritional value, flavor, and taste improve, but their natural defense mechanisms against pathogens, susceptibility to physiological disorders, and potential life span decrease. Therefore, for direct delivery to local markets, harvest should occur when the highest eating quality is reached. Early harvest is more appropriate for delivery to distant markets to prolong the postharvest life span (Crisosto & Valero, 2008). The fruit is harvested based on peel color breakage (Barbera et al., 1992b), fruit size, and fullness and flattening of the floral cavity or receptacle (Cantwell, 1995). According to Parish and Felker (1997), scar diameter is more helpful than scar depth to determine fruit maturity, as it is determined by sugar content. However, peel color breakage is the main harvest index used at the commercial level. Purple and deeply colored fruit varieties need to be harvested at less than 50% color break to have optimum quality (Temagoult et al., 2017).

In order to identify the best harvest time, objective and subjective maturity indices have been developed to include; the percentage of peel color break, approximate fruit weight of 110–160 g, and total soluble solids (TSS)  $\geq$  13% (Felker et al., 1997), pulp firmness (measured with an 8 mm plunger)  $\geq$  0.8 Newton mm<sup>-2</sup> (Pimienta-Barrios, 1990; Barbera et al., 1992b), reducing sugar level around 90% of that of fully ripe fruit. However, in some cultivars, the reducing sugars never exceed 50% of the total sugars (Pimienta-Barrios, 1990), abscission of glochids, flattening of the floral cavity of the receptacle, percentage of pulp, and peel thickness, ease of removal and resistance to physical damages (Cantwell, 1995). Timing for harvest is

an essential factor that determines fruit quality. For example, Schirra et al. (1999a) observed no differences in TSS, acidity, or pH with ‘Giulla’ cultivar harvested twice a year, in summer and autumn, whereas ascorbic acid content was higher in summer-harvested fruit, while fruit weight was higher in autumn-harvested fruit.

### 2.3 *Harvest Methods*

Cactus pears are particularly difficult to harvest because of the presence of glochids and spines, which can pierce the skin and enter the eyes and respiratory tract. Therefore, the fruit is harvested in the morning when the temperature is mild, and humidity is sufficiently high to prevent glochids from dislodging and floating in the air and prevent fruit dehydration. Pickers should be provided with protective clothing, gloves, and safety glasses. Despite the plant’s tough appearance and ability to withstand harsh environmental conditions, the fruit is very tender and cannot withstand rough treatment (Wessels, 1992). For most cultivars, the articulation’s physiological loosening connecting the fruit to the mother cladode is low at harvest time, and injury at the stem end is inevitable if the harvest has carried by snapping, pulling, or twisting. Therefore, for commercial purposes, a knife must be used to make a sharp cut at the fruit base with a small piece of cladode left attached, which is naturally detached later (Ochoa et al., 1997; Cantwell, 1999). Abd El-Ghany et al. (2015) developed a pruning shear (Fig. 10.1) to pick the fruit, and results showed a 7.2% increase in labor performance with reduced harvest cost.

### 2.4 *Postharvest Physiology*

It is essential to understand the nature of cacti fruit growth and preharvest biochemical changes to understand fruit postharvest physiology before proceeding with fruit postharvest handling and processing technology. Preharvest physiochemical changes include increased weight, diameter, pulp content, TSS, and vitamin C, along with a reduction in total acidity. By the 11th week, sugars increased about 100-fold in both peel and pulp, but the major sugar accumulation occurs by the end of the 6th week of fruit development. Glucose is generally the highest sugar, followed by fructose and sucrose, representing 80% of the TSS. The fruit is non-climacteric, known for its low respiration rate ( $20 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) with low ethylene production ( $0.2 \text{ } \mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$ ) at  $20 \text{ }^\circ\text{C}$ , and hence their physiological activity is low (Corrales Garcia et al., 1997; Yahia, 2012). Respiration rate declines during fruit development and is not different for fruit harvested at different stages of ripening. The fruit is not sensitive to external ethylene exposure, but exposure at warm temperatures may enhance yellowing (Moreno-Rivera et al., 1979; Cantwell, 1995; Schirra et al., 1997). Therefore, the perishability of the fruit is mainly related to physical damages rather than fruit physiology.

**Fig. 10.1** Fruit harvest tool (Abd El-Ghany et al., 2015)



The pulp's low acidity and high sugar content make the fruit very susceptible to microbial invasion and limit its storage life. The stem end is the leading site of infection due to wounding caused by harvest. In general, cacti fruit have a short shelf-life; for instance, under marketing conditions (20 °C and 60–70% RH), the fruit has a shelf life of only a few days due to decay, weight loss (WL), and softening (Rodriguez-Felix, 1991; Corbo et al., 2004). D'Aquino et al. (2014) reported that respiration rate declined gradually when fruit moved from hot field-temperature to warm temperature. Shelf life may reach up to 4 weeks under cold storage conditions (6–10 °C) due to reduction in respiration rate (Feugang et al., 2006); however, when fruit moved from cold storage to room temperature, respiration rate has increased again, in response to chilling temperature (Schirra et al., 1999b). Therefore, fruit marketing, long-term storage, and worldwide distribution are difficult (Kader, 2002).

Physical damages during harvest and transport can markedly compromise fruit quality and storage length and increase fruit susceptibility to physiological

disorders and decays (Piga et al., 1996). Bruises and wounds can occur due to finger pressure when cutting the fruit or impact when the fruit is dropped into the baskets and during handling and transportation. Injuries are also inflicted by cutting and glochids, which cause small rusty-brown discolored areas in the peel (Cantwell, 1995). Damage susceptibility varies between cultivar based on peel thickness and color; being green-colored fruit is more sensitive to discoloration (Cantwell, 1995; Pimienta-Barrios, 1994). The susceptibility of fruit to physical injuries increases with maturity, which favors microorganism growth, especially with mild temperature and high humidity during the autumn ripening season (second-crop fruit) (Potgieter & D'Aquino, 2017). A significant increase in ethylene production may also occur along with the increase in respiration rate in response to chilling temperature, pathogen infection, or physical damages (D'Aquino et al., 2014). No synthetic fungicide is registered for postharvest purposes; therefore, much care must be taken to avoid injuries and prevent microbiological decays that shorten fruit shelf life. The primary fungi that cause decay during storage are *Botrytis cinerea*, *Fusarium* spp., *Aspergillus* spp., *Penicillium digitatum*, *Penicillium italicum*, *Penicillium expansum*, and *Penicillium polonicum* (Chessa & Barbera, 1984; Rodriguez-Felix et al., 1992; Granata & Sidoti, 2002; Swart et al., 2003; D'Aquino et al., 2015; Faedda et al., 2015).

As a tropical-origin plant, the cactus pear is susceptible to chilling injury (CI) at a temperature lower than 5 °C, but some varieties are even sensitive when exposed for a prolonged period to temperatures below 10–12 °C (Ramayo et al., 1978; Corrales Garcia et al., 1997; Yahia, 2011). Potgieter and D'Aquino (2017) showed that a storage temperature range of 8–12 °C is recommended to prevent chilling injury, but this is affected by cultivar, geographical zone, preharvest environmental conditions, agronomic practices, crop type (summer or autumn crop), ripening stage at harvest and postharvest treatments. Chilling injury symptoms appeared on *Opuntia ficus-indica* 'Gialla' fruit after 14 days storage at 9 °C and 95–98% RH. Schirra (1998) reported the highest chilling injury sensitivity on 'Bianca' fruit among Italian grown cultivars. A 50% lower incidence of chilling injury was observed on *O. ficus-indica* fruit compared to *O. amyclaea* fruit grown in Mexico and stored for 15 days at 10 °C and 85–90% RH; while decay percentage was about 10% on *O. ficus-indica* and 30% on *O. amyclaea* (Ramayo et al., 1978). Differences in chilling injury sensitivity among cultivars have also been observed in Mexican cactus pears cultivars, such as Burrona, Cristalina, Amarilla Montesa, Picochulo, COPENA T-5, and COPENA Torrejoja. No chilling injury symptoms were observed on Burrona and Cristalina after 30 days of storage at 9 °C (95% RH), plus 6 days at room temperature and 65–75% RH, while a high chilling injury incidence (25–100%) was observed for Amarillo Montesa, Picochulo, COPENA T-5 and COPENA Torrejoja (Corrales Garcia et al., 1997). On the other hand, *O. ficus-indica* grown in Chile are reported to be relatively less sensitive to chilling injury, and they could be stored at 0 °C for up to 2 months (Cantwell, 1995). Differences in chilling injury sensitivity have been observed during storage at 6 °C and 90–95% RH, plus 3 days at 20 °C and 75% RH, based on harvest season; being summer ripening fruit are more sensitive to chilling injury and WL, but less sensitive to decay than autumn

ripening fruit. It has also been reported that fruit at the stage of color break is more sensitive to chilling injury and less sensitive to decay than the ripe one. The ripe fruit is less susceptible to chilling injury than the less ripe fruit. Symptoms of chilling injury on fruit peel are scalded or bronzed areas varying in size and intensity, or black-brownish pits and sunken brown spots that can appear at non-chilling temperature (D'Aquino et al., 2012, 2014). In addition to visible peel symptoms, chilling injury can also cause metabolic imbalance, which may alter the respiratory metabolism, induce anaerobic respiration and the production of undesirable volatiles (e.g., acetaldehyde, ethanol) and ethylene, and lower the overall host defense mechanism to pathogens (Schirra et al., 1999a; D'Aquino et al., 2014). As a result, chill-injured fruit may show peel disorders without any change in eating quality or chemical composition, or they may show minor symptoms of chilling injury at the end of the cold storage period but become highly susceptible to decay when moved to a warm temperature (Schirra et al., 1996, 1997, 1999b; D'Aquino et al., 2014), as shown in Fig. 10.2 (El-Saedy & El-Naggar, 2009).

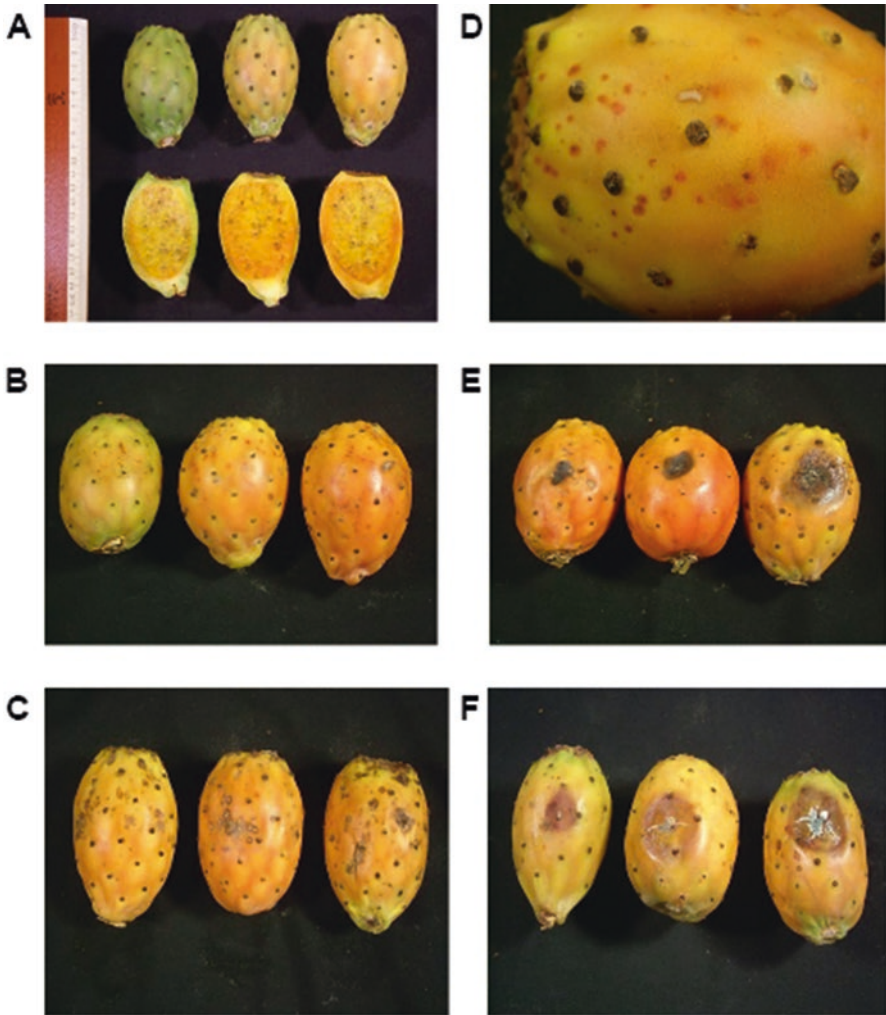
Preharvest treatment with gibberellic acid ( $GA_3$ ) or calcium chloride ( $CaCl_2$ ) does not affect or increase fruit susceptibility to chilling injury (Schirra et al., 1999a, b). Preharvest application with  $CaCl_2$  (2%) either 10 or 4 weeks before harvest, retarded peel color breakage at harvest (ripening), and reduced decay but increased chilling injury sensitivity of cactus pear, especially for summer crop stored at 6 °C and 90–95% RH with 3-days extended marketing period at 20 °C (Schirra et al., 1999a). A reduction in fruit decay during cold storage has also been observed on cactus pears preharvest treated with  $GA_3$  (10 ppm), but this treatment was ineffective on decay incidence during marketing at 20 °C for 3 days (Schirra et al., 1999b).

As a non-climacteric fruit, cactus pear does not contain starch. Therefore, after harvest, TSS, sugars, and organic acids decrease gradually depending on storage conditions, ripening stage, and cultivars (Corrales Garcia et al., 1997). Similarly, vitamin C content at harvest is about 10–80 mg 100 g<sup>-1</sup> based on the cultivar (Butera et al., 2002; Kuti, 2004), growth season (Sumaya Martinez et al., 2011), and ripening stage. It continuously increased even when the fruit is overripe (Coria Cayupan et al., 2011). Under cold storage conditions (6–10 °C), the vitamin C content is quite stable, despite the relatively high pH of the juice (Schirra et al., 1996), but it declines rapidly when fruit moved to room temperature (20 °C) (D'Aquino et al., 2014).

## 2.5 Postharvest Technology

### 2.5.1 Handling Procedures

Appropriate postharvest handling procedures are required to reduce transpiration and respiration rates and improve fruit postharvest life and marketability. The presence of spines and glochids is one of the main constraints limiting cactus pear consumption and marketability. Therefore, pickers usually collect the fruit in plastic baskets or lugs, and then spread them on the grass, and left them for curing in



**Fig. 10.2** Chilling injury symptoms on stored cactus pear fruit. (a) Initial quality of cactus pear fruit, (b) initial symptoms of chilling injury of the control fruit stored at 5 °C, (c) advanced chilling injury symptoms of the control fruit stored at 5 °C, (d) red spots on the control fruit stored at 10 °C, (e) decay incidence on chill-injured spots of the fruit stored at 5 °C, and (f) advanced decay incidence on chill-injured spots of the fruit stored at 5 °C (El-Saedy & El-Naggar, 2009)

ambient conditions to cure wounds and allow glochids loosening, and then the fruit is brushed with brooms (Cantwell, 1995), although brushing adversely affect keeping quality and increase the rate of water loss and decay (Testoni & Eccher Zerbini, 1990). Fruit should be quickly packed into plastic boxes holding 15–20 kg of fruit and transported for processing lines on the same day. If the fruit is handled a few days after harvest, they can be stored at ambient conditions for more curing or

pre-cooled in storage rooms at 6–10 °C to reduce transpiration and fungal attack (Wang, 1994; Potgieter & D'Aquino, 2017).

According to Barbera et al. (1992a), Bunch (1996), Piga et al. (1996), Mizrahi et al. (1997), Yahia (2012), and Potgieter and D'Aquino (2017), the following handling steps are followed for small-scale packing line with limited postharvest treatments:

1. Dry dumping before passing onto a series of rollers.
2. Brushing tunnel; a series of brushes, each rotating in the opposite direction for additional glochids removal, which is either vacuum-sucked out of the unit and deposited in a disposable bag or left to drop beneath the rollers.
3. Large round rotating table for sorting, sizing, grading, and packaging.
4. Fruit storage in a cool or refrigerated area.
5. Transportation to destination market.

Whereas, for large-scale harvesting, the fruit passes through the following steps:

1. Dry dumping before passing onto a series of rollers
2. Moving to conveyer belt where workers pre-select fruit (sorting by hand to remove damaged, spoiled, overripe, green, discolored, and misshapen fruit)
3. Brushing tunnel; designed with the same criteria as for a small-scale packinghouse.
4. In some countries (e.g., South Africa), the fruit is waxed to replace the natural waxes lost with glochids removal to reduce transpiration and enhance skin gloss.
5. Sizing; fruit pass through a mechanical or electronic device to classify them into different sizes.
6. Grading; in Italy, fruit can be sorted based on:
  - (a) Cultivar: peel shades can range from green to orange-yellow for the yellow cultivar 'Gialla', from green to ruby-red for the red cultivar ('Rossa'), and green straw-white for the white cultivar ('Bianca').
  - (b) Category (EXTRA and I)
  - (c) Weight (class B [105–140 g], class C [140–190 g] and class D [190–270 g]).
7. Packing; fruit is commonly packed according to color, size, and condition in ventilated plastic crates, 4.5 kg cartons, or single or double-layer tray cartons. Larger fruit can be packed in one-layer plastic nest trays inserted in a carton or plastic trays or directly in carton trays. Small fruit generally destined for local markets are packaged in plastic trays or punnets containing 6–8 fruit.
8. Cooling and storage; fruit should be cooled to 5 °C to reduce the loss of visual appearance (shiny surface) due to water loss. They are commonly room-cooled but may also be forced-air cooled. Cooling may be delayed if fruit undergoes a curing treatment. Fruit can be maintained for 2–5 weeks at 5–8 °C and 90–95% RH, depending on variety, ripening stage, and harvest season. Factors that limit fruit storage life include decay, water loss, and CI.
9. Transportation; to the destination markets in refrigerated conditions (7 °C). They can be transported alone or other commodities, by truck, ship, or aircraft.

### 2.5.2 Postharvest Treatments

Due to the high worldwide demand for fresh cactus pear fruit and extending the market window beyond the harvest season, and delay fruit deterioration, appropriate postharvest treatments are required to reduce transpiration and respiration rates and increase fruit tolerance chilling temperatures and prevent pathogen attacks. Fruit can be maintained for 2–5 weeks at 5–8 °C and 90–95% RH, based on the cultivar, ripening stage, and harvest season (Yahia, 2011). While refrigeration is the main means of prolonging the postharvest life of fresh fruit, the fruit's susceptibility to CI and decay, especially when moved from cold storage to warm temperature, has increased. Some other postharvest treatments are used to reduce fruit susceptibility to CI and decay, such as:

1. Curing at 38 °C for 24, 48, and 72 h in a vapor-saturated environment controlled fruit decay and improved their sensitivity to chilling temperature (Schirra et al., 1997). Fruit curing at 38 °C and 75–80% RH hastened the cladode piece's detachment attached to the fruit at harvest and improved the stem end scar (D'Aquino et al., 2014). High curing temperature (52 °C) delayed fruit ageing and WL, possibly due to melting and rearrangement of the epicuticular wax layers with a consequent filling of micro-cracks separating wax platelets; the main fruit transpiration pathway (Schirra et al., 1999a; Lopez Castaneda et al., 2010).
2. Heat treatments using hot water, either as dip treatment at 50–55 °C for 2–5 min (Schirra et al., 1996, 2002; Rodriguez et al., 2005; D'Aquino et al., 2012) or by water brushing at 60, 65, or 70 °C for 30, 20 or 10 s, respectively (Dimitris et al., 2005) inhibited the growth of pathogens that are naturally presented on the fruit surface. Hot water and vapor heat or hot air have also reduced the CI sensitivity of different horticultural commodities (Lurie, 1998). Schirra et al. (1999b) reported that postharvest heat treatments of 'Gialla' fruit, from summer or autumn crop, with hot water dipping (5 min at 55 °C) or hot air treatment (38 °C and 95% RH for 24, 48 or 72 h), followed by storage at 6 °C and 90–95% RH for 3–7 weeks, and additional 3–7 days at 20 °C were effective reducing the rate of CI and decay incidence, as well as WL, and maintaining fruit quality during storage. Additional protection against decay has been achieved with either hot air treatment at 37 °C and 90% RH or hot water dipping at 52 °C for 3 min, which could be attributed to the partial melting of the epicuticular wax that covers the gaps and micro-wounds on the fruit skin.
3. Controlled atmosphere (CA) storage (2% O<sub>2</sub> and 2–5% CO<sub>2</sub>) at 5 °C delayed loss of freshness and extended storage life of Italian cactus pears fruit to about 45 days (Testoni & Eccher Zerbini, 1990). Galletti et al. (1997) stated that stored cactus fruit under CA (2% O<sub>2</sub> and 5% CO<sub>2</sub>) at 0 °C showed the lowest decay incidence at 21 days and maintained sensory analysis for 42 days.
4. Modified atmosphere packaging (MAP); Brito et al. (2009) stated that MAP for light green cactus pear was efficient in delaying ripening and extending postharvest life up to 18 days storage at 10 °C and 92% RH using 12 µm polyvinyl chloride (PVC) film for modified atmosphere conditions. The fruit was firmer



and showed lower WL and less CI and decay symptoms than those stored under non-MAP conditions.

5. Intermittent warming (Chessa & Schirra, 1992) of 10 days at 2 °C followed by 4 days at 8 °C caused a reduction in CI on 'Gialla' fruit during storage for 6 weeks at 2 °C, followed by 1 week at 20 °C, compared with continuous storage (Schirra, 1998).
6. Film wrapping (Piga et al., 1996, 1997; Shumye et al., 2014), although it may induce anaerobic conditions and a build-up of undesirable volatiles like acetaldehyde and ethanol (Piga et al., 1996) if film permeability to gases does not match the oxygen (O<sub>2</sub>) requirement of packaged fruit. Film wrapping of 'Gialla' cactus pears delayed loss of fruit freshness and reduced chilling injury incidence during cold storage at 6 °C. This reduction in CI of packaged cactus pears was related to reducing WL (Piga et al., 1996).
7. Fruit coating using some natural or synthetic products as an alternative to synthetic film wrapping. The coating can be applied as a dipping or spraying treatment to the fruit and can also be combined with CA or MAP treatments (Sivakumar et al., 2016). Among these products is chitosan that exhibits an excellent gas barrier, delays change in color and total phenol content, and reduces the activity of browning enzymes like polyphenol oxidase (PPO) (Zhang & Quantick, 1997). Peeled prickly pear fruit submerged in chitosan solution mixed with acetic acid (1%) and stored for 16 days at 3–5 °C and 80–90% RH showed a delay in WL, softening, and color change (Ochoa-Velasco & Guerrero-Beltrán, 2014). Coatings based on sodium alginate, agar, and fish protein gel did not affect yeasts and mesophilic bacteria but stimulated the lactic acid fermentation, psychrotrophic, and coliform bacteria (Del Nobile et al., 2009).
8. Salicylic acid dipping treatment of 2 mM significantly decreased CI and retained quality of 'Taify' cactus pear during storage (Al Qurashi & Awad, 2012).
9. Experiments conducted with postharvest fungicides approved for other commodities revealed various degrees of effectiveness on cold-stored cactus pears, although these are not suitable for organic fruit production:
  - (a) Benomyl, captan, or vinclozolin treatments were ineffective in controlling postharvest decay (Gorini et al., 1993).
  - (b) Imazalil (IMZ) and thiabendazole (TBZ) treatments prevented natural decay induced by *Penicillium* spp., *Botrytis cinerea*, and *Alternaria* spp. in first-crop 'Gialla' fruit over a 2-month storage period at 8 °C, followed by 1 week of simulated marketing conditions at 20 °C; CI symptoms were also reduced. Sodium orthophenilphenate (SOPP), either alone or in combination with TBZ or IMZ, was phytotoxic, resulting in increased decay and WL (D'Aquino et al., 1996). The efficiency of TBZ was markedly increased when applied at 52 °C, even at a concentration 6 times lower than that applied at 20 °C (Schirra et al., 2002). The combined application of hot water with TBZ (dipping in 1000 ppm hot TBZ solution [55 °C] for 5 min) provides an alternative way to reduce the applied dose of this fungicide (from 1000 to 200 mg L<sup>-1</sup>) against decay (Schirra, 1998).

- (c) Fludioxonil, a synthetic fungicide registered over the last decade to control a wide range of decay-causing fungi in different commodities, very efficiently controlled cactus fruit decay when applied at 20 or 50 °C before storage; however, its effectiveness was reduced when applied at the end of cold storage (D'Aquino et al., 2015).

### 2.5.3 Processing

Cactus pear has many bioactive components that need to be preserved during processing. The high content of TSS and low acidity also makes fruit pulp a very attractive medium for pathogen growth, which requiring thermal treatment at 115.5 °C or higher to control micro-organisms (Yahia, 2011).

#### Fresh Cut (FC)

Fresh-cut fruit is very perishable because wounds caused by processing operations stimulate respiration and ethylene production, hastening the loss of cell components, softening, and senescence. Damaged tissues are also subject to oxidative browning due to PPO activity (Beaulieu & Gorny, 2004). The high demand for FC cactus pear has had a marked impact on companies involved in processing and distribution. More attention is paid to hygienic requirements, and new packing solutions are adopted to meet logistics and consumer requirements (Timpanaro et al., 2015). To produce a high-quality product, harvest at the optimal maturity stage is required; because when the fruit is overripe, peeling and slicing before packaging causes the mucilage to drip. The product then becomes less visually attractive, and microbial growth is promoted. Fruit must be of high quality, and conditions must be hygienic. Besides, the temperature must be below 10 °C during processing and constant during refrigerated storage. Moreover, the correct type of plastic film must be used for packaging (Yahia, 2011).

The shelf life of FC cactus pear 'Gialla' was extended, and chemical and sensory attributes were maintained for 8 days by placing the FC fruit in polystyrene trays sealed with polyolefinic film and storing them at 4 °C (Piga et al., 2000, 2003). The FC fruit texture was maintained with storage in ethylene-vinyl acetate (EVA) film for 7 days at 5 °C (Saenz et al., 2001). Modified atmosphere packaging at 4–8 °C for up to 7 days was reported to reduce microbial spoilage of bacteria and yeasts (Corbo et al., 2004). Other studies on the whole or halved peeled *Opuntia amyclae* have been carried out using three film types: polyolefin, 19 µm thick (PO19), and co-extruded bi-oriented polypropylene (25 and 35 µm). Whole or halved fruit could be stored at 4 °C for up to 20 days without any quality loss. Maintaining FC inadequate plastic film resulted in less WL, better fruit shine, and less ethanol production (Corrales Garcia & Sáenz, 2006). The whole peeled fruit immersed in citric acid as a preservative can be stored at 5 °C for up to 14 days with good microbiological and

quality characteristics. Citric acid does not affect the organoleptic quality (Oyarce, 2002).

Coatings based on sodium alginate, agar, and fish protein are not common at a commercial level and have no clear and consistent benefits against the microbial population (Palma et al., 2015). Anorve et al. (2004a) reported that peeled 'Cristalina' cactus pear fruit stored at 2 °C under CA conditions (3% O<sub>2</sub> + 10% CO<sub>2</sub>) retained excellent visual quality after 10 days of storage. Piga et al. (2003) suggested that CA storage inhibits the synthesis of phenolic compounds and the formation of condensed polyphenols that cause tissue darkening. Increased phenolic content was directly influenced by temperature and was linked to color changes, suggesting an association between phenolic content and color. The best temperature to preserve fruit and avoid darkening for up to 12 days was 2 °C (Anorve et al., 2004b). Storage response may also be affected by the stage of ripening and ripening season. The summer crop's overall quality declines faster than that of the scozzolatura crop (Allegra et al., 2015).

### Dried Fruit

Dehydration of cactus pear fruit is another means to produce edible processed products (Russell & Felker, 1987). The main factor influencing the drying process is the air temperature (Lahsani et al., 2004), reaching 60–70 °C. Drying rates decreased by increasing the cactus pear layer's thickness and were faster at 70 °C than at 60 °C (El-Samahy et al., 2007). Dried cactus products are healthy and could be prepared by blending with other fruit pulps and preservatives. For small-scale production, these products are easy to be prepared and involve low-cost technologies. Solar energy could also be used, especially in rural areas (Yahia, 2011). Dried cactus pear sheets of 'Taifi' cultivar have been prepared with the addition of sucrose, citric acid (to taste like traditional apricot sheets), olive oil (for softness), and sodium metabisulphite (to improve color) (Ewaidah & Hassan, 1992). Fruit leather made with cactus pear and quince pulp (75:25) has been prepared using dehydration in a forced air tunnel until moisture level decreased to 15–16% to maintain softness (Sepulveda et al., 2000). Attractive colored cactus pear bars have been prepared by mixing with apple pulp and flax seeds to increase fiber content (Lisham, 2009).

### Juice

The technology for cactus pear fruit juice production is more complicated than that for acidic fruit and less delicate flavors and aroma. Special control of pH and the duration and temperature of heat treatment is required since these factors are critical for juice preservation and final quality (Saenz & Sepúlveda, 2001). Pasteurization of juice produced from green pulp needs to be different from the colored pulp because betalain is more stable under heat treatment than chlorophyll (Sivakumar et al., 2016). Preservation technologies need to produce juice similar to fresh juice

(Rodriguez et al., 2015). In Egypt, El-Samahy et al. (2007) prepared juice using orange-yellow cactus pear, where the pulp was mixed with sugar solution (1:1) and adjusted before thermal treatment at TSS = 15 and pH = 5. This juice was filled into 100 mL glass bottles after heating to 80 °C and then pasteurized in boiling water for 25 min (the temperature inside the bottle was 95 °C). Juice bottles were refrigerated at 8 °C for 6 months. After storage, juice sensory characteristics were acceptable with low total bacterial count and absence of yeasts, molds, and coliform groups (Gurrieri et al., 2000; Jambi, 2017).

Concentrated juice is another means of juice processing that provides clear protection against microbial growth and can extend the juice's shelf life compared to regular juice. Concentrated juices are processed at a temperature close to 40 °C to reach up to 63–67°Brix (Saenz, 2000). The juice's stability against microorganisms' growth was good with relatively low sensory characteristics (Saenz & Sepúlveda, 2001). Concentrated juice of purple and red cactus fruit can be used after dilution, or they could be used for food coloring to increase antioxidants intake (Madrigal-Santillan et al., 2013; Sivakumar et al., 2016). Concentrated purple or yellow cactus pear juices have been used to color raspberry yoghurt (Saenz et al., 2001) and citrus juice (Moreno-Alvarez et al., 2003), respectively. Concentrated juice could also be used to produce other types of products such as vinegar (by acetic fermentation), syrups, toppings, nectars, and liquid sweeteners (Sivakumar et al., 2016).

Spray drying of *Opuntia streptacantha* juice has been carried out to produce dehydrated juice powder. The product maintains a very attractive color, but its high hygroscopicity makes it challenging to re-hydrate and dilute, with a significant loss of vitamin C (10.2%) compared to fresh juice (23.6%) (Ruiz-Cabrera et al., 2004).

## Frozen Pulp

Freezing is one of the most promising technologies for producing high-quality pulp that can be used (when diluted) to prepare liquors (by alcoholic fermentation), refreshing drinks, pastries, ice creams, jams, and sauces. This technology is not found on a large scale globally, except in California (USA), where a red cactus pear pulp sweetened to 22–24°Brix (Sivakumar et al., 2016). Bunch (1996) reported a stable frozen purée made from purple cactus pear with a percentage of pineapple juice, used in some beverages and food dishes.

## Peel and Seed Oil

Peel and seeds are the waste materials of the cactus pear processing industry; they can make up 40–60% and 10–15% of the whole fruit, respectively, depending on the cultivar (Russell & Felker, 1987). They can be used as a potential source of edible oil production; however, the oil yield is low and could only be a reasonably good source of lipid production from a massive cactus pear industry (Sivakumar et al., 2016; El-Beltagi et al., 2019). Ramadan and Morsel (2003) reported a relatively low

oil percentage extracted from fruit peel (3.68%). Linoleic, oleic, and palmitic acids were the major fatty acids, comprising more than 75% of total fatty acids. Extracted seed oil is also relatively low (6–17%) compared to other commonly used oilseeds. It contains unsaturated fatty acids, an essential linoleic acid content, and a low level of linolenic acid, affecting its stability (Sawaya & Khan, 1982; Sawaya et al., 1983a). Seeds could also be used as a fiber source (54.2 g 100 g<sup>-1</sup> DW), mainly cellulose (El Kossori et al., 1998).

### Encapsulation

The stability of betalain and polyphenols is critical (Castellar et al., 2003; Khatabi et al., 2016) for using them as antioxidants and food colorants. Stability could be improved using microencapsulation technologies, such as spray drying. Microencapsulation is a technique whereby a biopolymer encapsulates a bioactive compound to protect it from oxygen, water, or other conditions and improve its stability (Desai & Park, 2005). This method is also used to change liquid solutions to powders for easy handling (Rodriguez-Hernández et al., 2005).

## 3 Harvest and Postharvest Technology for Cladode Production

Cladodes are the rapidly growing succulent stems of the prickly pear cactus, *Opuntia* spp. They are also known as cactus stems, cactus pads, cactus vegetables, or phylloclades, and also called ‘nopal’ or ‘nopalito’ in Mexico for all cactus plants belong to the genus *Piatyopuntia* and *Nopalea*. Cladodes are mostly water (92%), carbohydrates, including fiber (4–6%), protein (1–2%), minerals, mainly calcium (1%), and moderate amounts of vitamins A and C (Yahia, 2012).

### 3.1 Maturity and Harvest Indices

Prickly pear is a CAM plant (Pimienta-Barríos et al., 2000), and therefore opens its stomata during the night to fix CO<sub>2</sub> as malic acid, which is then converted into sugar during the day. Therefore, the acid content (0.1–0.5%) and the cladodes’ flavor may fluctuate significantly during the day, something to be considered for optimal harvest time (Yahia, 2012). The acidity of several nopal varieties decreases during the day in relation to exposure to light (Flores-Hernandez et al., 2004). Even when detached from the plant, the variation is noticeable since the cladode remains alive and photosynthetically active. Cantwell et al. (1992) reported pH values of 0.94% in the morning, decreasing to 0.47% in the afternoon. Therefore, Pimentel Gonzalez (2013) recommended harvesting nopal 2–3 h after sunrise. Acidity depends on

cladode age and cultivar; small cladodes (<10 cm long) are not CAM-active. The acidity of cultivars ‘Jalpa’ and ‘Morado Italiano’ is 0.43%; ‘Milpa Alta’ is 0.68%; and ‘Oreja de Elefante’ is 0.69%. Cultivars ‘Jade’ and ‘Negrito’ present low oxidation activity, another important trait for agro-industrial processing (Aguilar Sanchez et al., 2007).

Flat, oval, or round cladodes should be harvested 30–60 days after sprouting when they weigh 80–120 g and are 15–20 cm long (Flores Valdez, 1995). Cladodes are harvested based on size and can be small (<10 cm long) or medium (<20 cm long). In Mexico, cladodes are harvested when they are 15–20 cm long (weight 90–100 g); however, cladodes harvested before reaching a length of 10 cm with an average weight of 30 g are usually CAM inactive and virtually lack spines. Overmature cladodes are thick with spongy white tissue, acidic in flavour, are not commonly consumed, but they are used for animal feed. Good-quality cladodes are thin, fresh-looking, turgid, and have a brilliant green color (Cantwell et al., 1992).

## 3.2 Harvest

Tender nopals can be harvested during the early stages of growth until reaching the light green color stage. They can be regularly harvested at an interval of 15–20 days (10–15 cm in length and 30 g in weight). The plant can provide an average yield of 1.5 kg plant<sup>-1</sup> year<sup>-1</sup> under greenhouse conditions (Kumar et al., 2018). Latex gloves and knives are required to harvest tender nopals for protection against glochids and mucilage. Mucilage loss and brown discoloration from the cut stem end is commonly a potential quality problem. The tender pads are carefully removed by inserting the knife between the joints of the cladode with the ‘mother cladode’—an operation requiring skill to maintain the product intact (Mondragon-Jacobo & Gallegos, 2017) with less physical damages to avoid water loss, quality reduction, and pathogen attacks. In any case, the harvest is typically early in the morning when humidity is the highest and workers’ exposure to glochids is reduced.

## 3.3 Postharvest Technology

### 3.3.1 Handling

After harvest, cladodes should be deposited in baskets or plastic crates and transported to the packinghouse for postharvest handling procedures, as follow;

1. Glochids cleaning: using a sharp knife and gloves is still the most common method for the complete removal of glochids. Other tools, such as razor-blade-type knives, hollowed and sharpened spoons, and potato peelers, have also been used, but with less successful results. A laser machine was once proposed, but it

never passed the modeling phase due to its high cost and low efficiency. The cleaning machine based on rotatory knives and built from stainless steel has been introduced by Mexican inventors to clean large volumes and can be adjusted for different shapes and sizes of nopalitos. The machine can process  $\leq 40$  pads  $\text{min}^{-1}$  with an estimated wastage of  $<15\%$ . The same manufacturer produces a second machine designed to slice clean nopalitos into different shapes: dices or strips. On-spot glochids cleaning at marketing offers the consumer proof of freshness. This initiative has been tried successfully in many Mexican supermarkets, where the freshly cleaned product is offered sliced or diced nopal upon customer's choice (Mondragon-Jacobo & Gallegos, 2017).

2. Packing: depending on the market destination, the growers cut the cladodes' edges and pack them in cylindrical or rectangular bales (1 m height), wrapped in brown paper or polyethylene sheets. Small, medium, and large cladodes are stored according to market standards (Mondragon-Jacobo & Gallegos, 2017).

### 3.3.2 Cooling and Storage

Major factors limiting the storage life of cladodes are decay and dehydration. Cladodes are cooled to about  $5\text{ }^{\circ}\text{C}$  to reduce the loss of visual appearance (shiny surface) due to WL and reduce the respiratory rate and senescence process. They are usually room-cooled but can also be forced-air cooled. Hydro cooling should be avoided as it favors discoloration and decay in damaged areas, especially where spines have penetrated the surface (Yahia, 2012). Cladodes stored under ambient conditions rapidly lose their brilliant and shiny appearance, become dull-green, and may begin to yellow and shrivel due to WL. Storage life can reach up to 3 weeks at  $5\text{ }^{\circ}\text{C}$  and 2 weeks at  $10\text{ }^{\circ}\text{C}$  when polypropylene foil is used (Rodriguez-Felix & Villegas-Ochoa, 1997). *Nopalea cochenillifera* cladodes lost only 7% water after 12 days of storage at  $20\text{ }^{\circ}\text{C}$  and moderate RH. High RH (85–89%) proved to be disadvantageous for this species (Nerd et al., 1997). Storage temperature can modify cladode acidity; for example, chilled storage ( $5\text{ }^{\circ}\text{C}$ ) maintains or slightly increases acidity, while storage at room temperature ( $15\text{--}20\text{ }^{\circ}\text{C}$ ) decreases acidity. Acidity fluctuations affect the cladodes' flavor; therefore, processing and consumption are more important than the harvest time (Corrales Garcia et al., 2004). Cladodes are chilling sensitive when stored below  $10\text{ }^{\circ}\text{C}$ , and symptoms may appear after 3 weeks or less at  $5\text{ }^{\circ}\text{C}$ . Chilling injuries on cladodes may appear as a superficial bronzing or unattractive surface discoloration with increased susceptibility to decay, especially at the cut stem end (Yahia, 2012). Some discoloration due to CI can occur if cladodes are stored longer than 2 weeks at  $5\text{ }^{\circ}\text{C}$  (Cantwell et al., 1992). Ascorbic acid content decreased 20–40% after 7 days of storage at  $20\text{ }^{\circ}\text{C}$  (Rodriguez-Felix & Villegas-Ochoa, 1997).

### 3.3.3 Modified Atmosphere Packaging (MAP)

Decay at the cut stem end may be a problem if cladodes are stored for up to 2 weeks (Yahia, 2012). Bacteria of the genus *Leuconostoc*, *Bacillus*, *Pseudomonas*, *Micrococcus*, and *Ruminicoccus* were identified in the microflora of *O. ficus-indica* cactus stems in MAP (Guevara et al., 2003). Fungicide dips reduce postharvest decay of cladodes but are not used commercially (Yahia, 2012). Therefore, MAP generating an atmosphere with O<sub>2</sub> levels of up to 8% and CO<sub>2</sub> levels of up to 7% in passive MAP, or CO<sub>2</sub> up to 20% in a semi-active MAP under 5 °C storage conditions has been used to delay ripening and senescence, and extend the shelf life and maintain quality of the cladodes for 32 days. Using MAP decreased changes in texture and color, and contents of water, texture, fiber, and chlorophyll, and mold, yeast, and mesophilic aerobic microorganisms, count on cladodes. The delay in fiber and chlorophyll degradation was related to reducing cellulase activity, hemicellulase and pectinase, and chlorophyllase, respectively. These benefits are due to atmospheric modification rather than to humidity increase in the atmosphere (Guevara et al., 2003). However, elevated CO<sub>2</sub> levels ( $\geq 40\%$ ) may injure the cladodes. Moderate CO<sub>2</sub> (5–10%) may help reduce discoloration and other cut cladodes' visual defects (Yahia, 2012).

### 3.3.4 Processing

Cladodes are commonly used as broiled, blended with other vegetables, or as a juice. Some species like *O. atropes* have increased recently in Mexico because of their texture and pleasant smell. *Opuntia leucotricha* and *O. robusta* produce high-quality cladodes because the pericarp can easily be removed, do not fall apart during boiling, and do not release mucilage (Vigueras & Portillo, 2001). Different products from the cladodes also include bread, jelly, jam, wine, and flavor components (Guevara & Yahia, 2005); however, this requires reducing the presence of mucilage for best results. Mucilage is a common feature of *Opuntia* spp. and released in response to wounding. The amount of released mucilage depends on the variety and the cladode's age and stage of dehydration. Mucilage is also released during cooking, which usually involves boiling. It cannot be wholly removed from nopalitos (Trachtenberg & Mayer, 1987). According to Pensaba et al. (1995), various practices have evolved to reduce or mask its presence in the final preparation:

- Addition of dry oregano leaves, bay leaves, chopped onion stems, tomatillo husks, sea salt grains, sodium bicarbonate, lemon juice, corn husk, or garlic cloves, depending on the used nopalito recipe.
- Intermittent heat treatment by boiling and dipping the nopalitos in cold or chilled water.
- Addition of a few copper or silver coins during boiling, although this method is the least advisable due to the high risk of contamination.



- Scalding with common salt and oregano for 7 min. This method maintains good texture and color. When vacuum-packed, it could be stored for 3 months at 4 °C.

The most aggressive cooking methods have a negative effect on vitamin and mineral contents, reducing the functional properties and beneficial effects of nopalito consumption. The salt-oregano method reduces the presence of mucilage without affecting the typical green color of nopalitos. Scalding and brining are the standard treatments for large volumes of nopalitos used in the restaurant industry in Mexico, and they led to the popularization of nopalitos in the 1990s (Pensaba et al., 1995). The presence of mucilage derived from the cooking process has been found essential for the production of various products, especially in combination with other products, including candies, balsamic vinegar, cheese, and pharmaceutical products including cream, gel, shampoo, tablets, and syrups, as well as waterproof materials and paints (Saenz, 1995).

## 4 Harvest and Postharvest Technology for Animal Nutrition

The benefits of using cactus cladodes, low-quality fruit, and processed-fruit wastage to feed ruminant animals are well documented (Nefzaoui & Ben Salem, 2001; Ben Salem & Abidi, 2009). Plantations of cactus for fodder production (harvested) or as forage (directly browsed by livestock or wildlife) have been developed in Italy and North Africa since the mid-nineteenth century (Le Houerou, 2002).

### 4.1 Productivity

In general, productivity varies with inputs and cultivation systems. The producer must consider land availability and the economic value of inputs and outputs when deciding what system best suits a particular condition (Dubeux et al., 2017). Under rainfed semi-arid agro-ecosystems of northeast Brazil, cactus can reach high productivity of  $>50$  t DM ha<sup>-1</sup> year<sup>-1</sup> in an intensive cultivation system (160,000 plants ha<sup>-1</sup>) with a high level of manure (80 t ha<sup>-1</sup> year<sup>-1</sup>) (Silva, 2012). Under such conditions, a cactus orchard productivity of 20 t DM ha<sup>-1</sup> year<sup>-1</sup> is sufficient for 4–5 cows annually (Santos et al., 2000). Farias et al. (2005) reported productivity of 1.5 t DM ha<sup>-1</sup> year<sup>-1</sup> in low input systems with no weed control. Cactus intercropped with other crops often have diminished productivity, for instance, the productivity of 2.2–3.4 t DM ha<sup>-1</sup> year<sup>-1</sup> with 5000 plants ha<sup>-1</sup> intercropped with *Sorghum bicolor* (L.) Moench has been reported by Farias et al. (2000).

## 4.2 Quality

Cactus cladodes are rich in water, sugars, ash, and vitamins A and C, but they are low in crude protein and fiber contents (Batista et al., 2003b). They exhibit a high Ca-P ratio and are highly palatable. Nutritive value varies according to the season, agronomic conditions, and cultural practices (Nefzaoui & Ben Salem, 2001). Cladodes (age 1–3) are high in water during winter and spring (85–90%), less in summer (75–85%), and the younger the cladode, the higher the water content. The ruminant consumes large quantities of cladodes due to the low DM content, leading to diarrhea. Therefore, the diet should be supplemented with a fibrous source rich in N (Le Houerou, 1996). Cladodes contain high ash content (100–250 g kg<sup>-1</sup> DM) (Sawyer et al., 2001) and crude protein because most of the total N is in a soluble form (865 g kg<sup>-1</sup>). The older the cladode, the lower the crude protein (CP) content (Ben Salem et al., 2002). Calcium followed by K are the most abundant minerals in the cladodes, but Ca's availability to rumen microflora and the host animal is compromised by the high content of oxalates and the extremely high Ca-P ratio (Batista et al., 2003b). Cactus cladodes are high in carbohydrates (600 g kg<sup>-1</sup> DM), starch (75 g kg<sup>-1</sup> DM) and  $\beta$ -carotene (6.5 mg kg<sup>-1</sup> DM) (Ayadi et al., 2009). Mucilage is high in the cladodes of spineless (6–13 g kg<sup>-1</sup>) and spiny (6–14 g kg<sup>-1</sup>) cactus. Its concentration increases at least twofold in summer compared to winter. Mucilage reduces salivation in ruminants (Abidi et al., 2009a). Other soluble carbohydrate-rich feedstuffs, such as molasses, cause acidosis in the ruminant because they are low or free of mucilage. Carotenenes, titratable acidity, and carbohydrates increase during plant development, while protein and fiber decrease. Cladodes are also high in malic acid due to a CAM-based diurnal rhythm (Lila et al., 2004; Mohammed et al., 2004; Newbold et al., 2005).

Cactus cladodes contain phytochemicals with no apparent detrimental effects on livestock. Total tannins range between 21 and 42 g kg<sup>-1</sup> DM in mature and young cladodes of spineless cactus, respectively (Negesse et al., 2009). Also, total oxalate content varies between 60 and 120 g kg<sup>-1</sup> DM, respectively. Cladodes of spiny cactus are higher in oxalates (110–118 g kg<sup>-1</sup> DM) than spineless cactus (102–105 g kg<sup>-1</sup> DM) (Abidi et al., 2009b). These oxalates are insoluble, so that they have no toxic effect; however, it is known that insoluble oxalates form complexes with Ca and Mg, making them unavailable for rumen microflora and the host animal (Dubeux et al., 2017). Spiny and spineless cactus cladodes are low in saponins (2–5 g kg<sup>-1</sup> DM), total phenols (10–34 g kg<sup>-1</sup> DM) and condensed tannins (<1 g kg<sup>-1</sup> DM) (Ben Salem et al., 2002; Abidi et al., 2009b).

The fermentation potential of the cladodes of different species and cultivars of cactus has been evaluated. The digestion of organic matter leads to volatile fatty acids, ammonia, and different gases, mainly CO<sub>2</sub> and methane (Andreu-Coll et al., 2020). High gas production is a characteristic of soluble carbohydrate-rich forage, including cactus, and this varies based on the cultivar, season, and cladode age. In northern Brazil, high gas production from 24 h fermentation was the highest for 'Gigante' cultivar (210 mL g<sup>-1</sup> DM), followed by cultivar 'Miuda' (202 mL g<sup>-1</sup>

DM), and the lowest was for 'IPA-20' cultivar (195 mL g<sup>-1</sup> DM) (Batista et al., 2003a). In southern Tunisia, high gas production of spineless (*Opuntia ficus-indica* f. *inermis*) and spiny (*Opuntia amyoclaea*) cactus cladodes harvested in winter was 138 and 140 mL g<sup>-1</sup> DM, whereas in summer was 140 and 145 mL g<sup>-1</sup> DM, respectively (Abidi et al., 2009b). The amount of metabolized energy was evaluated in spineless cactus growing in Ethiopia and found to be 7.5–8.5 MJ kg<sup>-1</sup> DM; and hence this cultivar was not a good source of energy since the ideal metabolized energy should be about 10–13.6 MJ kg<sup>-1</sup> DM (Negesse et al., 2009).

Cactus is a promising option for alleviating drinking water scarcity in dry areas and during drought periods. The more consumed fresh cactus, the less water the animal drunk. The decrease in drinking water consumption by various sheep and other animals is 40–98%, depending on the proportion of cactus in the diet (Monteiro et al., 2014). There was a 59% decrease in drinking water in lambs receiving a diet composed of 43% fresh cactus (Gebremariam et al., 2006). In Ethiopia, lambs stopped drinking water when they consumed a 55% fresh cactus-containing diet. Cactus is often used to supplement low-quality forage, including straw and pasture vegetation. Its impact on diet intake is improved when associated with a protein source. Total DM intake in lamb receiving pasture hay and cactus was 26% higher than that in lamb fed on pasture hay alone (Tegegne et al., 2007). The replacement of energy sources, such as barley grain or ground corn (Costa et al., 2012) by cactus in sheep diets increased total DM intake by 6% and 25%, respectively. Replacing barley in concentrates with cactus increased total DM intake by 26% in growing goats (Abidi et al., 2009a).

Cactus supply has no adverse effect on diet digestibility and may improve it (Ben Salem et al., 2004). Soluble carbohydrate-rich feeds like molasses and cactus improve diet palatability and enhance rumen fermentation, usually leading to increased total DM intake and/or diet digestibility (Tegegne et al., 2007). Depending on the diet composition, cactus increased organic matter (OM) digestibility of the diet by 2–10% in cactus-receiving lambs, and this could be the result of improved rumen fermentation (Costa et al., 2012). Cactus-fed lambs receiving teff straw or pasture hay showed a decrease in the diet's digestibility due to the total tannin content of cactus, which have a great affinity with proteins, making them unavailable for rumen microflora and the host animal (Gebremariam et al., 2006). However, Costa et al. (2012) reported an increase of 13% in CP digestibility of lamb's diet under Brazilian conditions.

Similarly, Souza et al. (2009) recorded a high value of CP digestibility (77–80%) in bucks receiving *Cynodon* hay, cactus, and soybean hulls. No effect of cactus on fiber digestibility of the diet (Misra et al., 2006). The replacement of barley with cactus as energy supplements in the goat lamb diet did not produce significant changes in the intramuscular fatty acid composition of meat, but vaccenic acid was higher, positively impacting the human cardiovascular system (Abidi et al., 2009a).

### **4.3 Direct Browsing (Forage)**

Direct browsing occurs mainly in native plantations with both thorny and spineless cacti. It is currently practiced in a small number of countries (e.g., Mexico and Ethiopia), where livestock directly browses the wild cactus populations. In Ethiopia, camels and cattle can intensively browse spiny cactus, whereas, in human-made plantations, direct browsing is not recommended because it usually results in rapid damage to the stands. The most effective and low-cost option is grazing with an electric fence, where all the biomass in a row must be completely consumed before the livestock can access another row. The greatest danger of direct grazing is the loss and wastage because cladodes are only partially consumed; thus, it is avoided (Le Houerou, 2002).

Spines in the wild populations may be burnt directly before grazing, as practiced in Texas. Maltsberger (1991) found that 15 L of propane were required for 14 cows per day, and 8 work-hours were required to prepare feed for 200 cattle per day. Plueneke (1990) stated that labor and fuel costs to burn spines are significantly reduced when the plantation is in rows; however, some wastage as cattle step on burnt portions of cactus. Therefore, cut-and-carry is the most commonly used technique for cactus feeding. It prevents wastage and excessive grazing. Both spiny and spineless cactus cladodes can be harvested and transported to the barn, then chopped, mixed with other feeds, and spread in troughs. Spines must be burnt before chopping and feeding. Different types of choppers are available in the market. In North Africa, cactus chopping is done manually with knives. In Tunisia and Ethiopia, transportable, low-cost choppers are made locally and are propelled using human power. More sophisticated and motorized choppers are utilized in Brazil and Mexico. In addition to reducing wastage, cactus slices are more convenient for incorporating mixed diets (Dubeux et al., 2017).

### **4.4 Harvest (Fodder Production)**

Cactus harvest management must consider the interaction between harvest intensity, frequency, and timing because this affects cactus regrowth and maximizes production (Farias et al., 2000). In general, increased harvest frequency requires reduced harvesting intensity, and these two factors interact with plant population. The residual photosynthetic area after harvesting is critical to increasing plant regrowth. A cladode area index (CAI) of 4–5 is essential to increase cactus productivity. The larger the plant population, the higher the CAI, resulting in increased productivity when no other factors limit growth (Nobel, 1995). Every 4 years, harvesting showed no difference between preserving primary and secondary cladodes, whereas harvesting every 2 years required a less intense cut to preserve all secondary cladodes (Farias et al., 2000). The productivity increased with greater plant population ( $\leq 83,333$  plants ha<sup>-1</sup>) with higher organic fertilizer inputs, harvesting

every other year, and preserving primary cladodes (Souza, 2015). Cactus are usually harvested in the dry season when livestock feed is scarce. In more intensive production systems, cactus is considered a component of the livestock diet throughout the year, but the year-round supply of cactus is not common in most regions. Producers usually take advantage of the rainy season for pastures and rangelands, leaving cactus for the dry season. During the rainy season, the cladodes' moisture content increases, and there is more chance of pathogen incidence on the cut surface of cladodes, resulting in more disease problems (Dubeux et al., 2017).

## **4.5 Postharvest Technology**

### **4.5.1 Handling**

In contrast to other fodder and forage crops that require storage (e.g., hay or silage), cactus is a standing evergreen crop, which can be used year-round. The natural and probably most efficient way of using cactus is to cut the cladodes and feed them without any processing. Moreover, cactus is rich in water and plays a crucial role in arid environments as a replacement for drinking water (Dubeux et al., 2017).

### **4.5.2 Processing**

The processing techniques differ, based on the utilization of harvested cladodes, as follow:

#### **Drying**

Cactus drying is mainly practiced in Brazil and South Africa. Under certain circumstances (e.g., short harvest period, producing a commercial feed, or mixing the cladodes with other ingredients), cactus cladodes are chopped into small slices then air-dried, ground to produce a cactus meal with consideration to avoid too-fine meal that could transit too quickly in the gut of ruminant animals (Dubeux et al., 2017). Sun drying is recommended to avoid using expensive fuels that will increase production costs. Sometimes, cladodes are dried to reduce their high-water content (85–90%) since fresh cactus consumption leads to very wet animal-feces (Menezes, 2008). However, De Waal et al. (2013) stated that even when animals are fed with dried cactus, feces remain wet due to the presence of mucilage. Sun-dried and coarsely ground cladodes can replace a large proportion of lucerne hay in young lambs' diet (Einkamerer, 2008), considering the importance of cactus-based diets with high-quality N sources for lambs (De Waal et al., 2013). The feeding behavior of milking cows that consumed machine-sliced cacti is recommended to maximize the DM intake and avoid alterations in milk composition compared to the

knife-chopped one. Besides, the total mixed ration feeding strategy is recommended than separate concentrates to reduce selectivity by cows, leading to an imbalance between the diet offered and consumed (Da Silva Vilela et al., 2010).

## Silage

Under certain circumstances, it may be necessary to make silage from cactus cladodes when the production is concentrated in a short period (pruning) or when wet agro-industrial by-products cannot be stored for a long time. To make high-quality silage, lactic acid fermentation is necessary, requiring appropriate levels of moisture (30–40%) and sugar in a full anaerobic environment (Saenz, 2017). Cactus cladodes contain sufficient carbohydrates for good lactic acid fermentation, but the high-water content requires careful mixing with other materials like chopped straw or bran (Dubeux et al., 2017). A mixture of chopped cladodes (350 kg), olive cake (a by-product of olive oil mills, 400 kg), and wheat bran (250 kg) with a pH of 4.5 improved the average daily weight gain and meat quality of the lambs (Abidi et al., 2013).

## Feed Blocks

Large quantities of cactus fruit are not harvested in some countries due to low quality or labor costs; the over-ripened fruit attracts the Mediterranean fruit fly that can cause extensive damage to other fruit crops (Saenz, 2000). Therefore, farmers are encouraged to collect these fruit and incorporate them in feed blocks for livestock. Different formulas have been developed based on replacing molasses with cactus fruit (Dubeux et al., 2017). Incorporating cactus fruit as a feed block ingredient with an oat-vetch hay-based diet improved the voluntary intakes of heifers and ewes (Chermiti, 1998). Feed blocks, composed of cactus fruit (90 g kg<sup>-1</sup> DM), olive cake (367 g kg<sup>-1</sup> DM), wheat bran (243 g kg<sup>-1</sup> DM), quicklime (154 g kg<sup>-1</sup> DM), urea (73 g kg<sup>-1</sup> DM), and salt (73 g kg<sup>-1</sup> DM), improved the total intake of organic matter and fiber, digestibility and N retention of the goats (Ben Salem et al., 2003).

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**Part III**  
**Chemistry, Functionality and Health-  
Promoting Properties of *Opuntia* spp.**

# Chapter 11

## Chemistry and Functionality of *Opuntia* spp. Nopal Cladodes



Maryna de Wit and Herman Fouché

**Abstract** *Opuntia* cacti are ideal crops for arid regions since they can generate biomass under water-stress conditions. *Opuntia* spp. are important as food and feed resources, with the young cladodes being harvested as a vegetable crop (nopalitos). Consumers are nowadays more health conscious and as a result, the food industry produce new food types based on nopalitos. Cladodes have a high nutritional value due to its contents of minerals, proteins, dietary fibre and phytochemicals. The chemical composition depends on the type of specie, environmental conditions, maturity stage, harvest season and post-harvest treatment. The soluble dietary fibre may help reduce body weight and calcium contents improves bone density. The beneficial properties are related to the mineral, phenolics, vitamins, polyunsaturated fatty acids and amino acids. The medicinal properties of cladodes include prevention of chronic diseases such as atherosclerosis, cardiovascular diseases, cholesterol, diabetes, cancer, obesity and metabolic syndrome as they have anti-atherogenic, anti-hyperglycemic and anti-hyperinsulinemic properties. Wound healing properties of cladode extracts was also reported. This chapter deals with the chemistry, composition and functionality of both mature cladodes and nopalitos from different *Opuntia* spp.

**Keywords** Cladode stems · Nopalitos · Functional properties · Cosmetics · Nutraceuticals · Pharmaceutical

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## Abbreviations

AAE	Ascorbic acid equivalents
ABTS	3-Ethylbenzothiazoline-6-sulphonic
ADF	Acid detergent fibre
ADL	Acid detergent lignin
BMI	Body mass index
CAD	Coronary artery diseases
CAM	Crassulacean acid metabolism
DEE	Diethylether
DM	Dry mass
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DW	Dry weight
FRAP	Ferric reducing ability of plasma
FW	Fresh weight
GAE	Gallic acid equivalents
GIP	Glucose dependent insulinotropic
GIT	Gastro-intestinal tract
GLP-1	Glucagon-like peptide 1
HA	Hyaluronic acid
HDL	High density lipoprotein
IDF	Indigestible dietary fibre
LDL	Low density lipoprotein
MUFA	Monounsaturated fatty acids
n-3	<i>Omega-3</i>
n-6	<i>Omega-6</i>
NDF	Neutral detergent fibre
ORAC	Oxygen radical absorbance capacity
PO	Phenoxy radical intermediates
PUFA	Polyunsaturated fatty acids
QE	Quercetin equivalents
ROS	Reactive oxygen species
SDF	Soluble dietary fibre
SFA	Saturated fatty acids
T2DM	Type 2 diabetes mellitus
TA	Titrateable acidity
TDF	Total dietary fibre
TE	Trolox equivalents
UV	Ultraviolet

## 1 Introduction

The *Opuntia* genera boasts a rich history that spans over 8000 years. The plant's presence in early history can be traced back to around 1492 in the Hispaniola Island where it was introduced to the Spaniards by the natives. Since then, the plant has formed an enormous part of the ecology, culture and economy of the areas they inhabit (Ochoa & Barbera, 2017). One example is the presence of the plant in the Aztec civilization. From there, it was introduced by the Spaniards to the rest of the world. The plant was well received because of its morphological properties, being used as fences and decorations in gardens, for its edible fruit and health properties. Further expanded properties such as cochineal dye production and use as vegetables made the plant useful in new territories. Today, cactus pear cultivation is being developed in at least 18 countries, mainly in arid and semi-arid regions. More than 100,000 ha are being extended. These plantations do not include the naturalized plants or those grown for home consumption (Hernández García et al., 2020). Since the sixteenth century, prickly pear has been an important subsistence crop in countries in America, Europe, Africa and Asia. The largest commercial fruit producing countries are Mexico, Italy and South Africa, followed by Chile and Argentina. Other smaller producing countries include USA, Peru, Columbia, Bolivia, Israel, Egypt, Jordan, Tunisia, Libya, Algeria, Morocco, Greece, Spain and Portugal. Vegetable (nopalitos; young cladodes) production in Mexico covers about 12,000 ha of cultivation area (Hernández García et al., 2020).

## 2 History and Background

The Opuntoid cactus is classified as a succulent since the plants store pronounced amounts of water. These plants are characterized mainly by a shallow root system for rapid uptake of water, a thick waxy cuticle to prevent water loss and an alternative photosynthetic pathway, Crassulacean Acid Metabolism (CAM), for nightly uptake of atmospheric CO<sub>2</sub>, to reduce water loss. The Opuntoid cacti are thus ideal crops for arid and semi-arid regions, since they can still efficiently produce biomass under water-restricted conditions (Del Socorro Santos Díaz et al., 2017).

*Opuntia* spp. are very diverse and widely distributed. Mexico has the richest variation and at least 126 species with various degrees of domestication have been noticed. Domestication processes of Opuntias have led to grouping of wild species into (a) wildest species including *O. streptacantha* and *O. hyptiacantha*, and (b) semi-domestic species such as *O. megacantha* and *O. albicarpa*. The *O. ficus-indica* is classified as (c) a long domesticated crop. *O. ficus-indica* is proposedly a spineless cultivar derived from *O. megacantha* (a native species from central Mexico). Domestication had improved flavor, size, shape, pulp and seed texture, and quality, while cladodes underwent changes in shape, colour, flavor, texture, sliminess (mucilage content) and quality (Del Socorro Santos Díaz et al., 2017).

The world-wide distribution of *Opuntia* can be ascribed to its easy propagation and its economic importance. It is known as a health-promoting food. It is not only grown for its fruit, but also for its production of vegetables, the young cladodes harvested as nopalitos (del Socorro Santos Díaz et al., 2017). As already mentioned, other uses and importance of cladodes include its industrial uses as well as its medicinal properties. *Opuntia ficus-indica* and other *Opuntia* and *Nopalea* spp. are being employed as hosts for cochineal insects in the production of red dyes (Del Socorro Santos Díaz et al., 2017).

Cladodes are modified stems that replace the photosynthetic function of leaves. The inner part of the cladode consists of (is formed) by the chlorenchyma, where photosynthesis occur, while the innermost is formed by a white medullar parenchyma that is responsible for water storage. Both the chlorenchyma and parenchyma store mucilage. The *Cactaceae* family is characterized by its mucilage production. Mucilage is part of the dietary fibre and is a complex polysaccharide. In general, the cladodes are rich in mucilage, pectin and minerals (Ondarza, 2016).

Cactus stems (cladodes) are highly appreciated for their chemical compounds providing anti-inflammatory antioxidant, anti-carcinogenic and anti-mutagenic properties (Stintzing & Carle, 2005). Chemical composition includes phytochemicals, mineral, dietary fibre,  $\beta$ -carotene, amino acids and vitamin A. A distinctive feature of the cladodes is their acidic taste (because of organic acids produced by CAM). Because of increased interest of consumers in health properties of food and its dietary intake, new food types based on e.g. nopalitos, have been produced by the food industry (Del Socorro Santos Díaz et al., 2017).

### 3 Chemical Composition of Cladodes (Nopales and Nopalitos) and Factors Influencing the Composition

Cactus cladodes contain high fibre contents, including pectin, mucilage, lignin, cellulose and hemicellulose, which are beneficial for glucose and lipid metabolism (Ayadi et al., 2009). Cladodes also contain phenolic compounds like flavonoids and phenolic acids, which are responsible for antioxidant activity. Dietary fibre analysis usually include determination of the soluble dietary fibre (SDF) as well as the insoluble dietary fibre (IDF). SDF (mucilage and pectins) determination is based on dissolution in water, followed by ethanol precipitation, while IDF measures fibre as the sum of lignin, cellulose and insoluble hemicelluloses (Van Soest & Wine, 1967). Other fibre tests include the NDF (Neutral Detergent Fiber) analysis which observe the plant cell's structural components such as cellulose and hemicellulose (Van Saun, 2013). Acid Detergent Fibre (ADF) measures the indigestible plant material excluding hemicellulose. Acid Detergent Lignin (ADL) is used to measure the lignin fraction of ADF. Chemical composition includes phytochemicals, mineral, dietary fibre,  $\beta$ -carotene, amino acids and vitamin A.

Cladodes of *Opuntia* spp. contain minerals, proteins, dietary fibre (as already mentioned) and phytochemicals that all contribute to its high nutritional value. The main components of these spp. are water (80–90%), carbohydrates (3–7%), fibres (1–2%) and proteins (0.5–1%). Cladodes contain on average per 100 g, 64–71 g carbohydrates, 19–23 g ash, 18 g fibre, 4–10 g protein and 1–4 g lipids. Furthermore, 7.2 g lipids and waxes, 3.6 g lignin, 46 g cellulose and 48 g other polysaccharides are present (Ben-Salem et al., 2005). Stintzing and Carle (2005) reported 93% water, 4–6% fibre, 1–2% protein and 0.8–3.3% pectin, as well as minor compounds, namely phytochemicals (including phenolic compounds and flavonoids, carotenoids, chlorophyll, polyunsaturated fatty acids (PUFAs) as well as acetic, propionic and butyric short chain fatty acids). Ciriminna et al. (2019) ascribed the retention of the green colour of cladodes during solar drying to the retention of the labile chlorophyll, carotenoids (yellow lutein),  $\beta$ -carotene (orange) and  $\alpha$ -cryptoxanthin. Carotenoids content varies with variety, maturity and place of origin and was reported to be the main antioxidant in cladodes.

Glucose and galacturonic acid are the main sugars of *Opuntia* cladodes (Ginestra et al., 2009). In the study of the phenolic, flavonoid and antioxidant contents of nine Mexican *Opuntia* spp. cladodes flour, it was found that *O. undulata* had the highest phenolics (905  $\mu\text{g}$  GAE/g), *O. robusta* had the highest antioxidant capacity (ORAC = 738  $\mu\text{mol}$  TE/g), while isorhamnetin was the most abundant flavonol, except in *O. lindheimeri*.

Raw cladodes were reported by De Santiago et al. (2018a, b) to contain (g/100 g) the following: 93.7% moisture, 1.1% protein, 0.5% ash, <0.1% lipids, 4.6% carbohydrates, 1% SDF, 2.1% IDF and 3.1% total dietary fibre (TDF). Phenolic compounds in raw cladodes included 0.86 mg/100 g quercetin, 1.02 mg/100 g kaempferol, 10.6 mg/100 g isorhamnetin, 12.5 mg/100 g total flavonoids, 2.95 mg/100 g ferulic acid, 0.61 mg/100 g 4-hydroxybenzoic acid, 3.56 mg/100 g total phenolic acids and 16.1 mg/100 g total phenolics. Isorhamnetin are the most abundant phenolic compounds found in cladodes, followed by quercetin and kaempferol derivatives in significant lower amounts. Quercetin is a flavonol with strong antioxidant capacity. Ferulic acid was found to be the predominant phenolic acid. The detection and levels of these compounds are influenced by differences in species type, cultivar, maturity stage, place of origin, harvesting season and environmental conditions as well as post-harvest treatments (De Santiago et al., 2018a, b).

Variations in composition of cladodes from different species are indicated in Table 11.1 (summarized from Astello-Garcia et al., 2015; Del Socorro Santos Díaz et al., 2017).

The composition of the cladodes varies between cultivars because of edaphic influences such as cultivation season, cultivation site and the cladodes' age, e.g. the various stages of development (Ventura-Aguilar et al., 2017). Older cladodes have lower acid levels than younger cladodes. Younger cladodes also contain more phenols than older (bigger) cladodes. As mentioned before, the type and amount of fibre also differ between species and cultivars, climate (rainfall and temperature) as well

**Table 11.1** Chemical composition of cladodes

Specie	Region	Protein (%)	Fat (%)	Crude fibre (%)	Ash (%)	Phenolic acids (mmol/g)	Flavonoids (mmol/g)
<i>O. streptacantha</i> (wild)	Mexico	11.2	0.73	7.3	12.6	56.8 0.66–11.07 mg/g DW	18.0 4.92–5.74 mg/g DW
<i>O. hyptiacantha</i> (wild)	Mexico	11.0	0.80	6.5	15.1	33.4 5.39–6.14 mg/g DW	17.1 4.86–5.62 mg/g DW
<i>O. megacantha</i> (semi-domesticated)	Mexico	10.7	0.69	6.5	13.6	44.7 6.7–19.5 mg/g DW	16.8 3.2–5.62 mg/g DW
<i>O. albicarpa</i> (semi-domesticated)	Mexico	11.6	0.75	6.5	13.2	40.8 (5.83–18 mg/g DW)	17.2 (2.5–5.62 mg/g DW)
<i>O. ficus-indica</i> (domesticated)	Mexico Spain	11.2	0.69	5.9	14.4	40.1 6.8–18 mg/g DW 128.8 mg/g FW	19.4 5.3–6.1 mg/g DW
<i>O. humifusa</i>	Mexico	4.7	1.25	50.3	2.0		
<i>O. robusta</i> (wild)	Mexico	17.4–19				0.39–2 mg/g DW	3.8 mg/g DW
<i>O. leucotricha</i>	Mexico					3 mg/g DW	1.8 mg/g DW
<i>O. stricta</i>	Reported only for fruits						
<i>O. undulata</i>	Mexico					0.95 mg/g DW	
<i>O. dillenii</i>	Spain					16.1 mg/g FW	
<i>O. violacea</i>	Mexico					20 mg/g DW	3.5 mg/g DW
<i>O. atropes</i>	Mexico					5.2 mg/g	9.7 mg/g
<i>O. rastrera</i>	Mexico					0.39 mg/g DW	
<i>O. lindheimeri</i>	Mexico					0.75 mg/g DW	

\*Cladodes from *O. violacea*, *O. megacantha*, *O. atropes* and *O. albicarpa* have high concentrations of phenolic acids (17.8–20 mg GAE/g DW), while *O. rastrera* and *O. undulata* have low values (0.39–0.95 mg GAE/g DW)

as age of the cladode. Spanish *Opuntia ficus-indica* young cladodes presented an important antioxidant activity by the ferric-reducing ability of plasma (FRAP) method as well as a higher total phenolic content (18.9 g gallic acid equivalent per kilogram) (Andreu et al., 2017). The authors also revealed the absence of sucrose and the presence of glucose and fructose, while citric, malic, and succinic acids were the main organic acids in all cultivars, with a significant higher content in old cladodes. Total phenolics are mainly characterized by isorhamnetin glycosides. The concentration of total flavonols ranged from 6.3 to 7.6 mg/g (DW) in the cladodes. Cladodes exhibited high total phenolic contents and antioxidant activities.

Eight South African cultivars from two *Opuntia* species were investigated for their antioxidant content and potential. The fresh fruit (pulp), peel, seeds and cladodes were compared in the study. When % DPPH\* (radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl) was tested, peel and cladodes were consistently the highest, while in the % chelating activity (Fe chelation) tests, fruit pulp and seeds



were the best tissue types. Cladodes contained more phenolics and carotenes than fruit. The cladodes of any cultivar contain similar and highly effective antioxidants (de Wit et al., 2019b; du Toit et al., 2018b).

In a study by López-Palacios and Peña-Valdivia (2020), who evaluated the phytochemical composition of young cladodes from 15 variants of *O. ficus-indica*, *O. albicarpa* Sheinvar, and *O. megacantha* Salm-Dyck (identified as being species with a highly advanced, advanced and intermediate degree of domestication, respectively), as well as *O. hyptiacantha* A. Web, and *O. streptacantha* Lem. (identified as wild-intermediate and wild species, respectively) it was found that out of the 13 identified and quantified phenolic molecules and terpenoids, only caffeic, ferulic and syringic acids, as well as the terpenoid  $\beta$ -amyryn were present in all variants. The flavonoid luteolin was absent in all five species. Gallic, vullinic, *p*-hydroxybenzoic, chlorogenic and *p*-coumaric acids were only present in 53–87% of variants, while the flavonoids quercetin, isorhamnetin, rutin and apigenin were found in 47–87% of the variants. Both oleanolic acid and peniocerol, were present in only 60% of the variants. Isorhamnetin was absent in *O. hyptiacantha* and quercetin in *O. streptacantha*. The differences and similarities in the secondary metabolites content showed no recognizable trend relating to the degree of domestication across the species in this genus. López-Palacios and Peña-Valdivia (2020) speculated and concluded that during the domestication process, the species of the genus *Opuntia* lose their ability to survive in the wild. The presence and concentration of secondary metabolites, which play a role in the interaction with their surroundings, are modified, but without a definite identifiable pattern. Secondary metabolites are a diverse group of bioactive compounds that can relate to a species evolution, both in their natural and domestication selection environments. In addition, these compounds are associated with plant resistance to stress when growing in the wild.

Keller et al. (2015) made the hypothesis that *Opuntia* spp. might protect the development of oxidative stress-associated diseases, such as atherosclerosis and colon cancer, *via* their antioxidant properties. They investigated the protective effect of *Opuntia* cladode powder against the oxidation of low-density lipoprotein (LDL) evoked by vascular endothelial cells, which is an important risk factor for atherosclerosis development, and the toxicity of 4-hydroxynonenal (a major lipid peroxidation product) on normal (Apc +/+) and pre-neoplastic (Apc min/+) immortalized epithelial colon cells. Various *Opuntia* species, classified according to their degree of domestication, from the wildest (*Opuntia streptacantha*, *Opuntia hyptiacantha*, and *Opuntia megacantha*), medium (*Opuntia albicarpa*), to the most domesticated (*Opuntia ficus-indica*) were tested. Cladode powders prepared from these *Opuntia* species significantly inhibited LDL oxidation induced by incubation with murine endothelial cells and the subsequent foam cell formation of RAW 264.7 murine macrophages and cytotoxicity on murine endothelial cells. Furthermore, *Opuntia* cladode powder blocked the promotion of colon cancer development on an *in vitro* model of colonocytes. The phenolic acid and flavonoids content, the antioxidant capacity, and the protective effect were relatively similar in all the cladode powders from wild (*O. streptacantha*) and domesticated *Opuntia* spp.

## 4 Nopalitos

Health benefits such as anti-inflammatory (Park et al., 2001), anti-ulcerogenic (Galati et al., 2003) antioxidant (Dehbi et al., 2013) and anti-cancer (Zou et al., 2005) of nopalitos are ascribed to high fibre, minerals, vitamins (A and C) and proteins (Gurrieri et al., 2000). The younger cladodes contain mostly carbohydrates (6.9 g/100 g dry matter), proteins and water. The pH is 4.6 and it has an acidity (titratable acidity, TA) of 0.45% (Stintzing & Carle, 2005). A cup of raw nopalitos contains 141 mg Ca, 19.8 µg vitamin A, 8 mg vitamin C, 4.56 µg vitamin K, 2.86 g carbohydrates, 1.89 g fibre, 1.14 g protein, 0.99 g sugar, 0.08 g fat, and 13.8 calories.

In a recent comprehensive study done by Makhalemele (2020) on the morphological and nutritional evaluation of nopalitos from 20 South African cactus pear cultivars (*Opuntia ficus-indica*), valuable information on a new food source to the South African consumer was found. Crude protein of 113.7 g/kg DM was found. A high content of protein is usually associated with high quality nopalitos.

The NDF (Neutral Detergent Fiber) analysis report on the plant cell's structural components such as cellulose and hemicellulose (Van Saun, 2013). An NDF content of 217 g/kg DM was found (Makhalemele, 2020). Acid Detergent Fiber (ADF) measures the indigestible plant material excluding hemicellulose. A low value indicates high digestibility and nutrient availability, which is more desired than a high value. This means that as the ADF concentration increases in plants, the concentration of digestible energy decreases. The ADF content had an average of 88.7 g/kg DM. Acid Detergent Lignin (ADL) is used to measure the lignin fraction of ADF. Low values of ADL are most desirable as it indicates a softer nopalito. The presence of high amounts of lignin decreases the digestibility of plant cell wall material, the intake of the plant material by consumers, as well as the performance of these consumers after consumption of the plant material. This could be explained by the fact that nopalitos are harvested while still young, while the concentration of lignin increases in the cell wall as the plant matures, which often leads to a tough, stringy texture (Chaves et al., 2002). An average of 30.4 g/kg DM ADL was found (Makhalemele, 2020). Nopalitos are regarded as low-calorie vegetables because of their low fat and high water contents (Stintzing & Carle, 2005).

Values of fat content were below 1%, while the moisture content was close to 100%. The total percentage fat content averaged 0.61%. Total lipids extracted from the nopalitos showed that palmitic acid (C16:0), linoleic acid (C18:2),  $\alpha$ -linolenic acid (C18:3c9,12,15 (n-3)), oleic acid (C18:1c9), stearic acid (C18:0) and linolelaidic acid (C18:2 t9,12 (n-6)) contributed significantly to the total fatty acid content. The highest ratio of fatty acids found in the different nopalitos included polyunsaturated fatty acids (PUFA), followed by the saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). These fatty acids averaged 49.0%, 38.7% and 12.1%, respectively (Makhalemele, 2020). Abidi et al. (2009) investigated the various fatty acids found in cladodes and showed linoleic acid (34.8%), linolenic acid (32.8%), palmitic acid (13.8%) and oleic acid (11.1%) to have been the most abundant in cladodes. When those researchers compared the linoleic acid content of

the cladodes with that of various crops, it was found that the stems contained a fatty acid content similar to that of argan oil (29–41%), but lower than that found in soybean (53.3%) and barley (51.2%) (Charrouf & Guillaume, 2007; Abidi et al., 2009). *Omega*-3 and *omega*-6 are two major classes of PUFA (Van De Walle, 2018). They are regarded as essential fatty acids, that cannot be synthesized by the body and which are required for brain function and cell growth and are obtained through the diet (Di Pasquale, 2009). Examples of *omega*-3 fats are  $\alpha$ -linolenic acid, docosahexanoic acid and eicosapentaenoic acid. Only  $\alpha$ -linolenic acid was found in high levels in the nopalitos. The total *omega*-3 fatty acids had an average of 18.7%. Simopoulos (2002) has reported that humans have evolved over the years on a diet which primarily consisted of an *omega*-6 to *omega*-3 ratio (n-6:n-3) of approximately 1. An increased *omega*-6/*omega*-3 ratio have adverse reactions which may result in the promotion of pathogenesis of diseases such as autoimmune diseases, cancer and cardiovascular disease (Simopoulos, 2002). On the other hand, low *omega*-6/*omega*-3 ratios have shown to suppress negative effects (Simopoulos, 2002).

The minerals which were found in abundance in the nopalitos were calcium (Ca), potassium (K), magnesium (Mg), phosphorous (P) and sodium (Na), respectively (Makhalemele, 2020). Calcium, which is highly regarded for its ability to improve bone strength in humans, is essential for membrane function in plants, averaged 31.0 g/kg DM. Potassium, which is essential in maintaining the electrolyte balance in plant cells and the human body averaged 18.6 g/kg DM. Magnesium, required for the body to make proteins, averaged 12.8 g/kg DM. Phosphorous, essential for healthy teeth and bones, averaged 2.70 g/kg. Sodium, playing a role in maintaining the electrolyte balance in the body, averaged 0.38 g/kg DM (Makhalemele, 2020).

Of the three sugars investigated, D-glucose yielded the highest results and averaged 2.99 mg/g (Makhalemele, 2020). This could be due to the fact that since nopalitos are young stems, it is highly possible that they would not contain that high concentration of glucose, compared to older cladodes, since glucose levels in plants tend to gradually increase as plants grow. Naturally, vegetables have minimal amounts of glucose and fructose compared to fruits. The Australia New Zealand Food Authority (FSANZ, 1999) reported fructose values found in common Australian vegetables such as green beans (0.2 g/100 g FW), asparagus (0.8 g/100 g FW) and common peeled cucumber (1.1 g/100 g FW). These three common vegetables are often closely associated with nopalitos. Sucrose (made up of glucose and fructose) is the primary transport form of assimilates in plants (Ciereszko, 2018). This study had an average of 0.26 mg/g of sucrose (Makhalemele, 2020).

Starch is a polysaccharide that is made up of individual glucose chains and plays a role in storing energy in plants. The starch content of the various nopalito cultivars averaged 1.7 g/100 g (Makhalemele, 2020). The interesting relationship between starch and sucrose seemed to be noticeable in the obtained results. The results of the two may be ascribed to the starch sucrose interconversion that takes place in different developmental stages of plants (Owlgen, 2019). It is in this process, whereby the plant either synthesizes sucrose and converts it into starch, or synthesizes starch and converts it into sucrose. The synthesis and conversion of these carbohydrates are dependent on the needs of the plant at that specific developmental stage. From this,

the converted carbohydrates are then transported and stored into various tissues to fulfil various functions in the plant. The existence of an inverse correlation between the two types of carbohydrates, whereby one increases as the other decreases were observed, which supports the existence of the starch sugar interconversion in nopalitos.

The secondary metabolites in plants (polyphenols) serve many functions, such as strong antioxidant and anti-inflammatory effects, both of which can be beneficial for the prevention of cancer (Zhang & Tsao, 2016). The metabolites (which were investigated in this study) averaged 2.55 mg GAE/g (Makhalemele, 2020).

Good quality nopalitos are regarded as fresh-looking, turgid with a bright green colour (Guevara et al., 2001). The main chlorophyll compounds, chlorophyll-a and chlorophyll-b, were investigated in this study (Makhalemele, 2020). Chlorophyll-a has the pivotal function of being that of primary electron donor in the electron transport chain part of photosynthesis. The primary function of chlorophyll-b is to broaden the absorption spectrum of organisms so that they can absorb more energy from the higher frequency blue light part of the spectrum. Chlorophyll-b averaged 1.64 DEE mg/g in this study (Makhalemele, 2020). Accessory pigments facilitate the absorption of various colours on the light spectrum. One such example is the carotenoids which reflect orange, red and yellow light waves. In the leaves, they form clusters next to chlorophyll-a molecules to give off absorbed photons aptly. In this study, carotenoids averaged 0.84 DEE mg/g. Flavonoids are a diverse group of plant chemicals which are responsible for the bright colours in fruits and vegetables along with carotenoids (Szalay, 2015). In this study, the flavonoids found averaged 0.24 mg QE/g. The average vitamin C content was 130 mg/100 g (Makhalemele, 2020).

Antioxidant activity is defined 'as a limitation of the oxidation of proteins, lipids, DNA or other molecules that occurs by blocking the propagation stage in oxidative chain reactions' (Huang et al., 2005). Primary antioxidants directly destroy free radicals, while secondary antioxidants inhibit the formation of free radicals indirectly through Fenton's reaction (Atasoy et al., 2019). Ways in which this activity was measured in this study was through the Ferric Reducing Antioxidant Power (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) assays (Makhalemele, 2020). FRAP assay is a widely used method that uses antioxidants as reductants in a redox-linked colorimetric reaction, wherein which ferric iron ( $\text{Fe}^{3+}$ ) is reduced to ferrous iron ( $\text{Fe}^{2+}$ ) at low pH which causes the formation of a coloured ferrous-probe complex from a colourless ferric-probe complex. The average FRAP content was 1.73  $\mu\text{mol}$  AAE/g (Makhalemele, 2020). The ORAC assay depends on free radical damage to a fluorescent probe, of which fluorescein is the most commonly used probe (Ou et al., 2001). The damage comes as a result of an oxidising reagent which leads to a progressive loss of fluorescent intensity. There exists a correlation between the resulting damage and the amount of oxidant present. In this study, the average of the ORAC content was 89.4  $\mu\text{mol}$  TE/g (Makhalemele, 2020).

According to literature, the ideal commercial raw nopalito must be thin, turgid, fresh-looking appearance, have a bright green colour (Ruiz Pérez-Cacho et al., 2006) and preferably be low in acidity and mucilage content since they have a negative influence on the eating qualities of the crop (Razo & Sánchez, 2002). The

climate of the area of cultivation is one of the key factors that cause variation in the composition of nopalitos, while different years (seasons) also caused variation in the various attributes of the nopalito cultivars. In general, various significant differences were observed for the influence of cultivar on 20 attributes out of a total of 23 attributes (Makhalemele, 2020), with the exception of pH, compressibility, moisture content, % solids, % waste and viscosity. The influence of year on the various attributes also showed significant effects on 17 attributes out of a total of 23. Non-significant differences were observed for the weight, length, width, diameter, moisture content, % solids, nopalito weight, line-spread viscosity and °Brix measurements of the various nopalito cultivars. With regards to the influence of the cultivar X year interaction on the various attributes, significant differences were observed for 13 attributes out of a total of 23. It has further been reported that a large gradient exists among the various species of cladodes in the wild, which implies that the size of nopalitos is dependent on the species being observed. The level of mucilage found in nopalitos affects the eating qualities of the vegetable (Makhalemele, 2020).

The colour of fruits and vegetables is one the most critical attributes as it may assist in determining the health and ripeness of the crop. Furthermore, the colour may assist in determining the kind of nutrients which are present in those fruits and vegetables (Bruso, 2018). Green vegetables, such as nopalitos, are green in colour as a result of the chlorophyll present. Nutrients which are common in such green-coloured vegetables include vitamin A, vitamin C and lutein which is important for healthy vision (Bruso, 2018).

Compressibility measures how soft or hard plant tissue is. High values of this attribute indicate nopalitos which have a softer tissue, while low values indicate nopalitos which have harder tissue. Firmness indicates how flexible the nopalitos are. High values indicate thin and flexible nopalitos, while low values indicate firm nopalitos. pH measures the acidity of a substance. According to Cantwell et al. (1992), the pH of nopalitos is most likely to be approximately 0.94% in the morning, while it is most likely to decrease as the day progresses to approximately 0.47%. Therefore, it has been recommended by Pimentel-González et al. (2015) to harvest nopalitos 2 h after sunrise, since the acid content is still low. The pH of the various cultivars ranged between 4.01 and 4.25, with an average of 4.16 (Makhalemele, 2020). The level of acidity found in nopalitos affects the eating qualities of the vegetables. Too high levels of acidity presents sourness, which is regarded as undesirable by consumers. Titratable acidity is an important parameter in the determination of organic acids present in nopalitos. Jianqin et al. (2002) have reported that cladodes comprise of various acids such as citric, malic and oxalic acid which are all affected by climate, soil and storage conditions (Cantwell et al., 1992). Significant differences were observed for the effect of cultivar on acidity. The titratable acidity ranged between 0.15% and 0.57%, with an average of 0.37% (expressed as citric acid) (Makhalemele, 2020).

The moisture content of fruits and vegetables vary significantly, e.g. between raw cucumbers (96%) and melons (92–94%) having high contents of water, while the likes of green lima beans (67%) and avocados (65%) have lower water contents.

The moisture content of the 20 nopalito cultivars ranged between 77.5% and 91.7%, with an average of 86.6% (Makhalemele, 2020).

Seasonality refers to the regular change in environment, as well as the biological responses acclimatised by that environment (Battey, 2000). Cactus pear plants are well adapted to grow and survive the harshest of conditions where there is immense water stress and poor soil. Nopalitos are regarded as a crucial re-vegetation crop to regulate water and erosion in areas which are under environmental duress (Pimienta-Barrios & Nobel, 1994). The colour of nopalitos is perceived to be green, however, great variation exists in the various cultivars' intensity of this attribute. It has been reported that the chlorophyll content and environmental aspects such as water availability, micro- and macro- nutrients and irradiance are the main determinants of colour in nopalitos (Irizar-Garza & Peña-Valdivia, 2000; Nobel, 2001). Significant differences were observed in the different colour coordinates of the nopalitos over the 2 years (Makhalemele, 2020). The timing at which nopalitos should be harvested is an important factor, seemingly because harvesting seasons vary according to the agroclimatic conditions, the variety of cultivars being harvested, as well as whether coerced blooming was utilised as a production technique (Berger et al., 2013). The differences of this attribute in the nopalito cultivars may be a result of the time at which the nopalitos are harvested, the age, management as well as the area in which the nopalitos were produced (López-Palacios et al., 2010; Meraz-Maldonado et al., 2012).

Harvesting month and other environmental and agronomic factors affect the composition of cladodes, e.g. *Opuntia ficus-indica* from different countries. The maturity stage also affects their biological properties. Flours of small and medium sized cladodes had higher contents of dietary fibre, viscosity, water absorption and swelling. These different fibre contents and viscosities might affect the *in vitro* and *in vivo* glucose responses (Nuñez-López et al., 2013).

## 5 Effect of Processing on Chemical Composition

Culinary processes such as microwaving, griddling and frying increased both soluble and insoluble fibre, while griddling and microwaving increased the flavonoid and phenolic acids up to threefold (De Santiago et al., 2018a, b). Boiling had a detrimental effect on the nutritional profile of cladodes (De Santiago et al., 2018a, b). All cooking methods also increased the antioxidant capacity measured by the DPPH<sup>•</sup> method. Antioxidant capacity measured by the ABTS (3-ethylbenzothiazolone-6-sulphonic) method also showed increased capacity in processed cladodes.

Normally, dietary fibre (pectin and mucilage) can form complexes with various bioactive compounds, rendering them unavailable. Thermal processes could increase their bioavailability (De Santiago et al., 2018a, b). These could be due to the hydrolysis of the cell walls and sub-cellular compartments, leading to the release of dietary fibre-bound polyphenols, while thermo-hydrolyzation of flavonoid glycosides can induce the formation of novel compounds such as Maillard reaction

products (e.g. melanoidins) which also contribute to sensory properties as well as antioxidant capacity.

An n-6/n-3 ratio of close to 1 will indicate protection against degenerative pathologies (as already explained), as well as pathogenesis of cardiovascular disease, cancer and autoimmune diseases, and as such, the significant correlation between MUFA intake and reduced inflammation, risk for coronary heart diseases and improvement of endothelial function is of importance when frying cladodes in olive oil (De Santiago et al., 2018a, b). A total of 45 phenolics were quantified in cladodes by De Santiago et al. (2018a, b). Flavonoids (isorhamnetin) and phenolic acids (eucomic) increased with microwave cooking and griddling. In another study by De Santiago et al. (2018a, b) 27 phenolics were identified and quantified, with piscidic acid being most abundant.

In a recent study by Du Toit et al. (2018) the antioxidant content and capacity of fresh and processed cladodes were determined. It was found that all cultivars demonstrated high antioxidant capacities and consequently, the cladodes of any cultivar could be considered as suitable for processing or preserved to produce healthy products. Regarding fruit color, the highest antioxidant content and capacity were found in cladodes from orange and purple fruit-bearing cultivars. Processing had a greater influence on the antioxidant content of cactus cladode products than the influence of cultivar. Dried products were the best in terms of antioxidant content and capacity. Processed cladodes, more than fresh cladodes, from all the cultivars, were concluded to provide an excellent source of antioxidants and could be suggested for products such as cladode flour and pickles. Based on the data, the following recommendations can be made: dried products made from cladodes of any cultivar/color as well as pickles from cladodes of any cultivar. Ascorbic acid levels in dried cladodes were the highest (182–282 mg/100 g) and the lowest in preserves (14.3–28.0 mg/100 g). In this study the highest carotene content was found in dried orange fruit-bearing cladodes (254.7 µg/g) and the lowest in orange fruit-bearing chutney (1.36 µg/g) samples. The values in dried cladodes were exceptionally high (103.8–254.7 µg/g) compared to fresh cladodes (6.72–17.8 µg/g). In fact, the carotene content in fresh cladodes was only 7% of the value in dried cladodes. This is due to the concentrating effect of the drying process. Reasons for the observed increase in carotene content after processing was summarized by Rickman et al. (2007) as higher extraction efficiency, release of protein-bound carotene during heat treatments, degradation of oxidative enzymes and the loss of soluble solids. The values of all other products were statistically similar; in fact, it was very similar to fresh cladodes. The high phenolic content found in fresh cladodes (42.8–270.9 mg/kg) was also seen in processed cladode products, in fact, dried cladodes had phenolic contents similar to that of fresh values (181.1–273.4 mg/kg). The phenolic levels for chutney (104.9–124.8 mg/kg), juice (73.1–102.3 mg/kg) and pickles (91.8–147.1 mg/kg) did differ significantly from fresh and dried cladodes.

The beneficial properties of the *Opuntia* spp. can be related to its contents of compounds such as minerals, vitamins, amino acids, PUFA and phenolics. In particular, bioactive compounds include phenolic acids, flavonoids, betalains and vitamins (Del Socorro Santos Díaz et al., 2017).

Cladodes are good sources of dietary fibres which, because of binding of these fibres to dietary fat, and increasing its excretion, may help in reducing body weight (hypolipidemic). High content of calcium in cladodes improve the bone mineral density in women with low bone mass. The soluble dietary fibre and Ca contents are important elements for the physical properties of the cladodes, since the main cell wall polysaccharide are made out of low methoxyl pectin. The cladodes also contain strong viscous components and hydrophilic polysaccharides with high molecular weights that provide them with the capacity to absorb and retain water (Del Socorro Santos Díaz et al., 2017). Calcium has an important role in the retention of water in cladodes (Stintzing & Carle, 2005).

Du Toit (2015) reported a mean content for Ca in mucilage powder of 3.01% of the mucilage ash content. Many researchers reported high contents of Ca in cladodes, e.g. values of 3.4 g/100 g (Sáenz, 1997) and 35.3 mg/g (de los Angeles Aguilera-Barreiro et al., 2013). Some researchers have also suggested the consumption of young cladodes (nopalitos) to increase calcium intake (Hernández-Urbiola et al., 2010; Morales et al., 2012; Moßhammer et al., 2006; Rodríguez-García et al., 2007; Russell & Felker, 1987; Sáenz, 1997, 2002). These results were consistent with the findings by Ayadi et al. (2009) who reported higher Ca values in spiny cladode flour (3.03 g/100 g DM) than in spineless (1.4 g/100 g DM). In cladodes, 5.64 g/100 g (dry weight) (Feugang et al., 2006) and 5.64–17.5 mg/100 g (El-Mostafa et al., 2014) were reported. El-Safy (2013) found 287–1335 parts per million. Ramírez-Moreno et al. (2012) found that young cladodes could be a source of Ca for people unable to eat dairy products and for people in developing countries. Cladode flour made from cladodes that were 40–135 days old had ~15 to ~35 mg/g Ca that showed a positive correlation with cladode maturity (Hernández-Urbiola et al., 2010). Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 1.35% and 3.3% Ca which also increased with maturity (Rodríguez-García et al., 2007).

Calcium oxalate crystals probably occur inside vacuoles. Vacuoles are known as the dumping ground and a warehouse, as excessive substances can move in and out depending on demand. Excess Ca that would be toxic to cytoplasm is absorbed into vacuoles where it becomes trapped in the form of calcium oxalate crystals (Salisbury & Ross, 1992). The calcium oxalate was mostly detected in the insoluble dietary fibre fraction of the cladode. The Ca content in soluble fibre was higher and calcium carbonate levels were 50% higher in soluble fibre. The bioavailability of Ca in cladodes were determined through calculating the oxalate:calcium ratio, as the ratio was  $\geq 1$ , calcium would be bioavailable in cladodes (Rojas-Molina et al., 2015). The presence of calcium oxalate crystals in the fresh cladode tissue has been reported by many authors (Contreras-Padilla et al., 2011; Ginestra et al., 2009; McConn & Nakata, 2004; Saenz et al., 2012; Trachtenberg & Mayer, 1982). Druse crystals were abundantly present in the epidermis and palisade layer in the three *O. ficus-indica* cultivars (Algerian, Morado and Gymno-Carpo), while in Robusta cultivar (*O. robusta*) the crystals were present but not as abundant, in a different form and smaller (Du Toit, 2015).



The mean potassium % of total ash obtained from extracted mucilage was 2.75% (Du Toit, 2015). In cladodes, 2.35 g/100 g K (Feugang et al., 2006) and 2.35–55.2 mg/100 g K (El-Mostafa et al., 2014) were reported. Cladode flour made from cladodes that were 40–135 days old had 55–72 mg/g K that showed no correlation with cladode maturity (Hernández-Urbiola et al., 2010). Ayadi et al. (2009) reported 3.5 g/100 g DM K, while Stintzing and Carle (2005) concluded that 60% of total ash was potassium (166 mg/g).

Vegetables usually have phosphorous values of below 100 mg/kg, but mucilage powder maintained higher levels and had an average of 109.46 mg/kg (0.01%) (Du Toit, 2015). Cladode flour made from cladodes that were 40–135 days old had 0.2–0.4 mg/g phosphorous that showed no correlation with cladode maturity (Hernández-Urbiola et al., 2010). Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 0.29% and 0.38% phosphorous content (Rodríguez-García et al., 2007).

Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 5.52% and 6.84% sodium content that increased with cladode age (Rodríguez-García et al., 2007). Na average content in mucilage powder was 118.2 mg/kg (0.01%) (Du Toit, 2015). El-Safy (2013) reported 465 ppm of Na in cladode flour. In cladodes, 0.4 g/100 g (Feugang et al., 2006) and 0.3–0.4 g/100 g (El-Mostafa et al., 2014) was reported. Cladode flour made from cladodes that were 40–135 days old had 0.2–0.55 mg/g sodium that showed no correlation with cladode maturity (Hernández-Urbiola et al., 2010). Dehydrated cladodes between 60 g (22 days old) and 200 g (64 days old) had between 0.21% and 0.12% sodium content that decreased with cladode age (Rodríguez-García et al., 2007).

Ayadi et al. (2009) found that calcium, iron and magnesium values were high in cladode flour but that sodium, phosphorous, zinc and copper contents were present but low. El-Safy (2013) determined the mineral content of cladode flour and concluded that it was considered to be a good source of minerals and an important food for humans; especially the calcium, potassium and magnesium values were significantly high. In fact, it was stated that cladode flour or fresh nopalitos could serve as an alternative source of calcium when dairy products cannot be consumed.

The NDF and ADF together constitute the insoluble fibre content of freeze-dried mucilage (Du Toit, 2015). The low totalled NDF and ADF values showed that the mucilage that was extracted from cladodes were mostly purely soluble fibre (mostly mucilage and possibly pectin) and contained very little insoluble fibre.

Phenolic compounds are important antioxidants. The phenoxy radical intermediates (PO) act as terminators of the propagation phase of oxidation by binding to other free radicals. These PO's are relatively stable molecules due to resonance. Alternatively, the phenolic hydroxyl groups can either donate a hydrogen atom or a free radical causing radical scavenging activity. They can also delocalize an unpaired electron and thereby extend the conjugated aromatic system. Some phenolics may also prevent metal-induced free radical formation when these phenolic compounds contain dihydroxy groups that can conjugate transition metals (Del Socorro Santos Díaz et al., 2017).

A recent study with 15 *Opuntia* cultivars from *O. streptacantha*, *O. hyptiacantha*, *O. megacantha*, *O. albicarpa* and *O. ficus-indica* showed that domestication grade had no effect on the metabolite content. Differences depended on the characteristics of each species (Astello-García et al., 2015). More than 30 phenolic compounds were identified in cladodes from different species.

Cactus powder, as reported by Sáenz (2015) is a source of dietary fibre and phenols. The soluble fibre is a positive feature to be used as food ingredient. Processing of cactus pear (old and young cladodes) by traditional and new methods is of economic importance to subsistence rural farmers, commercial farmers/producers, as well as small, medium-scale and large scale enterprises. As already mentioned, cactus cladodes have been recognized for many years to contribute to a healthy diet and as a medicine. This is mainly due to the content of dietary fibre. In most countries, the daily intake of fibre is lower than the recommended allowances, and therefore the consumption of cladodes either as young cladodes (nopalitos), fresh or processed, or older cladodes (flour) could improve this deficiency. The phenols present in the cladodes also improve the health properties. Dietary fibre prevent illnesses and diseases such as gastro-intestinal disorders, and high blood serum cholesterol (Sáenz, 2015).

One of the main differences between old and young cladodes are the fibre type and amount. One year-old cladodes have lower crude fibre (12% DW) than older cladodes (4 years of age) (17.5% DM). Dried nopalitos (1–3 months old), however had 20.4% fibre. The ratio of insoluble fibre to soluble fibre might also differ. The properties of different types of fibre vary. Therefore, the content of mucilage will have an influence on sensory properties. An ideal dietary fibre should have a balanced composition between soluble and insoluble fractions, should be as concentrated as possible with a weak taste, colour, texture and odour (Larrauri, 1999). Technological processes to prepare food fibres should aim to reduce negative characteristics of the cladode flour (powder) such as the plant flavor and slimy (mucilaginous) texture in order to be used as functional ingredient.

## 6 Properties of *Opuntia* spp. in Chronic Diseases

### 6.1 Cardiovascular Diseases

Atherosclerosis and related cardiovascular diseases are the leading chronic disease causing death. Coronary artery diseases (CAD) risk factors include hypertension, high cholesterol, smoking, diabetes and obesity. Lifestyle changes focusing on dietary habits and smoking are becoming a priority and lead to increased interest in the nutritional benefits of *Opuntia* spp. to prevent CAD (Del Socorro Santos Díaz et al., 2017).

### 6.1.1 Cholesterol Lowering Properties of *Opuntia*

Consumption of dried leaves of *O. ficus-indica* caused an increase in circulation HDL cholesterol with a decrease in LDL cholesterol, indicating an hypocholesterolemic effect (lipid lowering properties). This might be due to the content of dietary fibre, e.g. pectin. Pectin and its mechanism may have an influence in alteration of hepatic cholesterol (Del Socorro Santos Díaz et al., 2017).

### 6.1.2 Anti-atherogenic Properties

Most antioxidants are anti-atherogenic since they neutralize the formation of reactive oxygen species (ROS) and therefore exhibit anti-inflammatory and anti-apoptotic properties against the effects of oxidized LDL. Cladode powders from *O. streptacantha*, *O. hyptiacantha*, *O. megacantha*, *O. albicarpa*, and *O. ficus-indica* inhibited LDL oxidation and formation of foam cells by macrophages, indicating an inhibitory effect on the early stages of atherogenesis. These powders also exhibit anti-inflammatory properties as well as inhibition of toxicity of cell-oxidized LDL. Powdered cladodes of *O. streptacantha* and *O. ficus-indica* reduced the development of atherosclerotic lesions and therefore cardiovascular diseases (Del Socorro Santos Díaz et al., 2017).

## 6.2 *Opuntia* spp. in Diabetes

*Opuntia* cladodes have been reported to have antihyperglycemic and antihyperinsulinemic properties. Ingestion of *O. ficus-indica* nopal improved the postprandial response of glucose, insulin, glucose-dependent insulinotropic peptide (GIP) index and the glucagon-like peptide 1 (GLP-1) index on Type 2 diabetes mellitus (T2DM) patients. This hypoglycemic mechanism was attributed to the dietary fibres pectin and mucilage that slow down the absorption of glucose by increasing the viscosity of blood in the GIT (Del Socorro Santos Díaz et al., 2017).

## 6.3 *Opuntia* spp. in Obesity

Adipogenesis is a process involving coordinated changes in adipocytes morphology, sensitivity and gene expression. Adipocytes are responsible for lipid homeostasis and energy balance by either storing triglycerides or releasing free fatty acids in a response to changes in energy demands. Obesity is caused by both adipose tissue hypertrophy as well as adipose tissue hyperplasia, triggering the transformation of pre-adipocytes into adipocytes. This adipocyte dysfunction is associated with the development of obesity. *Opuntia* kaempferol or isorhamnetin flavonoid can

suppress lipid accumulation or inhibit adipogenesis (Del Socorro Santos Díaz et al., 2017).

Animal models with diet-induced obesity as well as clinical trials provided many clues to the potential effects of *Opuntia* extracts in treatment of obesity. Anti-obesity agents from *O. ficus-indica*, e.g. a natural fibre complex, showed patients had a reduction in body mass index (BMI), body fat composition and waist circumference. *Opuntia*-derived fibres bind to dietary fat, increases its excretion, reduces its absorption and leads to lower energy intake and weight loss. This product also induced a decrease in blood glucose and could contribute to blood glucose management (Del Socorro Santos Díaz et al., 2017).

#### 6.4 *Opuntia* spp. in Cancer

Different parts of *Opuntia* plants have cytotoxic effects on cancerous cell lines. Cladode flour extracts were tested on two human colon cancer cell lines: apoptosis-resistant and apoptosis-susceptible. It was found that the extracts effects were related to apoptosis induction. Extracts from *O. humifusa* cladodes were also able to induce apoptosis in human colon cells. These extracts were reported to suppress the growth of glioblastoma cells. Protective effects of various *Opuntia* cladode flours against the cytotoxic effect of 4-hydroxynonenal on colon cancer cells were also reported (Del Socorro Santos Díaz et al., 2017). The effect of *O. ficus-indica* cladode extracts on oxidative stress and genotoxicity induced by mycotoxins such as zearalenone and aflatoxin B1 were reported.

#### 6.5 *Opuntia* spp. in Skin Wound Healing

Wound healing can be affected by various pathological conditions, e.g. diabetes, arterial diseases, metabolic diseases, aging and infections, as well as by local and systemic factors, e.g. hypoxia, oxidative stress, decreased immunity, infection agents, inflammatory cytokines, metallo-proteases ...etc. Nopal extracts were and are used as traditional medicine for the healing of burns, wounds and skin disorders. Cladode extracts were shown to protect the epidermal barrier and the keratinocyte functions by upregulating the expression of two proteins (filaggrin and loricrin) present in differentiated keratinocytes and corneocytes. ROS production caused by inflammatory agents are inhibited (Del Socorro Santos Díaz et al., 2017).

The cicatrizing properties of *O. ficus-indica* cladodes might include both higher molecular weight polysaccharides namely a linear galacton polymer and a branched xyloarabinan, together with low molecular weight components including lactic acid, D-mannitol, piscidic acid, eucomic acid and 2-hydroxy-4-(4'-hydroxyphenyl)-butanoic acid. These components fasten cell regeneration on scratched keratinocytes monolayers implying *O. ficus-indica* components to exhibit wound healing and anti-inflammatory properties (Del Socorro Santos Díaz et al., 2017).

*O. humifasa* cladode extracts were reported to regulate the production of hyaluronic acid (HA) by increasing HA synthase expression during keratinocytes when exposed to UV-B radiation. Cladode extracts from *O. humifasa* could decrease the UV-B induced expression of hyaluronidase indicating that *O. humifasa* cladode extracts have strong skin care capacities (Del Socorro Santos Díaz et al., 2017).

Cactus plants have been used for many centuries by Native Americans as a dietary supplement and traditional folk medicine. Cladodes have been and are still used as therapeutic agents to treat gastric ulcers and for healing. Antiviral activity of cladodes extracts against herpes, HIV 1 and influenza A viruses have been reported. Hepatic toxicity caused by the organophosphorous insecticide chlorpirifos was reduced by *O. ficus-indica* cladode extract, while hepatoprotective effects towards benzo(a)pyrene as well as aflatoxicosis, were observed. Anti-cancer activities and chemopreventative activities of *Cactaceae* plants were reported. Cactus flour (dehydrated cladodes) are commercially available as nutraceutical supplements. It is sold as ingredients of food products such as baked products, cereal bars ...etc. Nopal capsules are believed to contribute to weight loss due to the fibre binding to dietary fats, and reducing its absorption. A marked decrease in cholesterol and triglycerides (hypercholesterolemic), as well as hypoglycemic effects were noted in either patients, mice and rats (Nazareno, 2015).

## 7 Cladodes as Functional Food

Functional compounds are “those that help prevent disease” (Ondarza, 2016). These compounds include fibre, hydrocolloids, pigments, minerals and vitamins, such as vitamin C (ascorbic acid), with antioxidants properties. When these compounds are included in foods, these foods or beverages are known as functional foods providing physiological benefits.

Cladodes are an important source of fibre, Ca and mucilage (Saéñz et al. 2006). *Opuntia* cactus has also been recognized as a source of nutraceuticals—“a food or part of a food that provides medical and health benefits, e.g. by prevention and/or treatment of a disease” (Ondarza, 2016). These nutraceuticals include mucilage, pectins, vitamins and phenolics. Soluble fibres (pectin and mucilage) and some hemicelluloses have hypolipidemic, hypoglycemic and hypocholesterolemic effects and have been used to treat obesity (Saéñz et al. 2006).

Dehydrated cladodes is a functional ingredient employed in functional food and to formulate nutraceutical and cosmetic products (Patel, 2014). IDF provides benefits to the GIT and reduce therefore risks of diabetes, obesity, coronary heart disease and certain cancers. Mucilage and pectin (cell wall) soluble fibre are hydrocolloids binding water by absorbing and concentrating water molecules at their surface and influence (increase) viscosity (Ciriminna et al., 2019). It was reported by Du Toit et al. (2019) that mucilage could replace unwanted ingredients in functional food products. Cladode powder was also added to wheat flour to improve the total phenolics content and antioxidant potential of bread (Msaddak

et al., 2017) as well as to baked products to increase dietary fibre content and reduce gluten content (de Wit et al., 2015). Functional food and health-promoting properties and applications of cladodes mostly include fibre-rich foods, weight-control products and dietary supplements (Ciriminna et al., 2019).

Skin-care and cosmetic products are formulated with cladode powder, since it protects the skin cells by firstly limiting the formation of free radicals, reduce the production of melanine caused by UV radiation and to limit the production of cytokins by epidermis cells (Patel, 2014), e.g. it was found that cladode powder led to 31% reduction in face wrinkle surface, 14% reduction in melanin formation (pigmentation) and 18% reduction in UV-induced oxidation along with an increase in the skin's immune defense system.

*Applications of cladodes and mucilage (from cladodes as summarized by Ondarza, 2016).*

- (a) Food and beverages: various food products as well as alcoholic and non-alcoholic drinks are made from the young cladodes (nopalitos), while livestock feed is produced from older cladodes.
- (b) Pharmaceutical industry: includes mucilage extracts for gastric mucosal protection in capsules and tablets with cladode flour and pectin for the capsules' production.
- (c) Cosmetic industry: creams, lotions, shampoos and sunscreens from cladodes.
- (d) Food supplements: e.g. flours and fibres from cladodes.
- (e) Natural additives such as gums (hydrocolloids) from cladodes.
- (f) Construction industry: using cladodes/mucilage as binding agents.
- (g) Energy sector: to use cladodes in digestion to produce biogas or burning of lignified cladodes as fuelwood.
- (h) Agricultural inputs: improved soils, organic material and drainage from the use of cactus pear products.
- (i) Tourist sector: artisan crafts made from lignified cladodes.

These different uses of the cladodes offer (especially for food or fodder) depending on the maturity (size and age) of the cladodes. Extraction of mucilage from the cladodes provide an important use of the cladodes. These hydrocolloids are especially used as a thickening agent with interesting viscosity properties. It can compete with other gums such as locust bean gum, guar ...etc. (Ondarza, 2016; du Toit et al. 2018a, b).

Mucilage can be used as a fat replacer and flavour binder. It was found that mucilage had an adverse effect on crystallization of ice crystals in ice-cream and sorbets; the formation of few large ice crystals instead of many small crystals prevented the products from freezing effectively. When freeze-dried mucilage is added to everyday products, such as yoghurt, as a bioactive agent, it would increase its positive physiological effects in the body and thereby redefine the product as a functional food product. Freeze-dried mucilage was successfully added to yoghurt after pasteurization and fermentation in order to increase the soluble fibre, as well as nutrients such as antioxidants and minerals. The addition of the mucilage powders could be increased and addition of flavourings added. No mucilage-containing functional

food products are commercially available in South Africa to the knowledge of the researchers. Mucilage addition had an adverse effect on crystallisation of ice-cream and sorbet, but could be used as fibre additive in yoghurt (de Wit et al., 2019a, du Toit et al. 2019).

The demand for functional foods is expanding as more consumers become obese or allergic to dairy, eggs and gluten. Functional food products could increase well-being, yet the undesired ingredients need to be replaced to restore the textural properties. Mucilage is a hydrocolloid gum, extracted from cactus pear cladodes that could transfer its function in plants to foods and be developed into a profitable functional ingredient. The investigation of the functional properties of extracted freeze-dried powders was necessary to predict the potential of South African cultivars as healthy ingredients. Native mucilage was applied in fat-, dairy- and egg-replacement products. Mucilage was successfully applied in mayonnaise products to replace up to 50% egg yolk and 30% oil. It was concluded that mucilage powders might contribute to the textural and nutritional quality of food products. The acceptance of mucilage powder as an active functional and nutraceutical food ingredient Mucilage showed promise as emulsifier and fat-replacer in mayonnaise products and was described as creamy with a highly acceptable mouthfeel. Mucilage is a natural, health improving product that the food industry could benefit from. It showed strong potential as a functional ingredient (Du Toit et al., 2019). It was concluded that mucilage powders might contribute to the textural and nutritional quality of food products. The acceptance of mucilage powder as an active functional and nutraceutical food ingredient will also lead to the development of cactus as a commercially viable crop in arid and semi-arid areas where few other crops can survive.

## 8 Medical Applications of Cladodes

*O. ficus-indica* is used for burns, wounds, edema, dyspepsia, as well as neuroprotective, cytoprotective, antispasmodic and chemopreventive (Galati et al., 2007). Anti-hyperglycemic effects are described to the fibre (mucilage and pectin) content. Some species such as *O. streptacantha* cladodes were used as anti-diabetic foods. Furthermore,  $\beta$ -sitosterol acts as anti-inflammatory agent. Mucilage has been reported to have wound healing activity (Carvalho et al., 2014), since it has the ability to retain water, therefore maintaining high humidity at the interphase of the wound. It also contributes to anti-oxidation as well as antibacterial activity.

## 9 Conclusions

*Opuntia* cacti are ideal crops for arid regions since they can generate biomass under water-stress conditions. In addition, cladodes have a high nutritional value due to its contents of minerals, proteins, dietary fibre and phytochemicals (antioxidants). The

**Table 11.2** Medicinal properties of *Opuntia* spp. cladodes (summarized from Nazareno, 2015)

Specie	Medicinal property
<i>Opuntia streptacantha</i> ; <i>Opuntia</i> spp.	Antiviral
<i>O. ficus-indica</i>	Anti-hyperlipidemic and cholesterol lowering
<i>Opuntia</i> spp.	Antiobesity
<i>O. monacantha</i>	Hypoglycemic and antidiabetic
<i>O. ficus-indica</i>	Anti-inflammatory
<i>O. ficus-indica</i>	Antiulcerogenic and anti-gastritis
<i>O. ficus-indica</i>	Alcohol “hangover” alleviation
<i>O. ficus-indica</i>	Anti-nickel-induced toxicity
<i>O. ficus-indica</i>	Anti-oxidative damage caused by zearalenone
<i>O. ficus-indica</i>	Diureticum
<i>O. ficus-indica</i>	Healing
<i>Opuntia</i> spp.	Cancer-preventative
<i>O. humifusa</i>	Bone density increase
<i>O. humifusa</i>	Insulin sensitivity increase

medicinal properties of cladodes include prevention of chronic diseases such as atherosclerosis, cardiovascular diseases, cholesterol, diabetes, cancer, obesity and metabolic syndrome, since they have anti-atherogenic, anti-hyperglycemic and anti-hyperinsulinemic properties due to its contents of these functional compounds present (Table 11.2). These chemical compounds are also responsible for the reported wound healing properties of cladode extracts. *Opuntia* spp. are important as food and feed resources, with the young cladodes being harvested as a vegetable crop (nopalitos).

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# Chapter 12

## *Opuntia ficus-indica* (L.) Mill. Bioactive Ingredients and Phyto-Constituents



Mohamed Fawzy Ramadan 

**Abstract** Consumer interest in novel food with high nutritional value has increased. *O. ficus-indica* is a plant that grows wild in the semi-arid and arid regions. The nutritional value and bioactive potential of *O. ficus-indica* fruit, fruit by-products (peel and seeds), and cladode and their use in the production of novel food products are promising. Owing to *O. ficus-indica*'s nutritional traits, the fruits and fruit by-products gain popularity as a gourmet diet. With high vitamin C, tocopherols, betalains, readily absorbable sugars, high mineral content, and essential amino acids, *O. ficus-indica* is tailor-made for novel food formulations. *O. ficus-indica* is usually consumed fresh, while peel and seeds are discarded as bio-wastes. *O. ficus-indica* by-products (peel, and seeds) and cladode contain phenolics, minerals, and dietary fiber, which could be used as functional ingredients. Rich in bioactive ingredients, *O. ficus-indica*, and plant by-products are anticipated to revolutionize the food production and solve food scarcity. The current chapter summarizes the scientific literature on the composition, nutritional value, and active constituents of *O. ficus-indica* fruit, fruit by-products (peel and seeds), and cladode.

**Keywords** Cactaceae · Cactus pear · Prickly pear · Mineral · Carotenoids · Betalains · Amino acids · Vitamins · Peel · Seed · Cladode · Pulp · Taurine

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## 1 Introduction

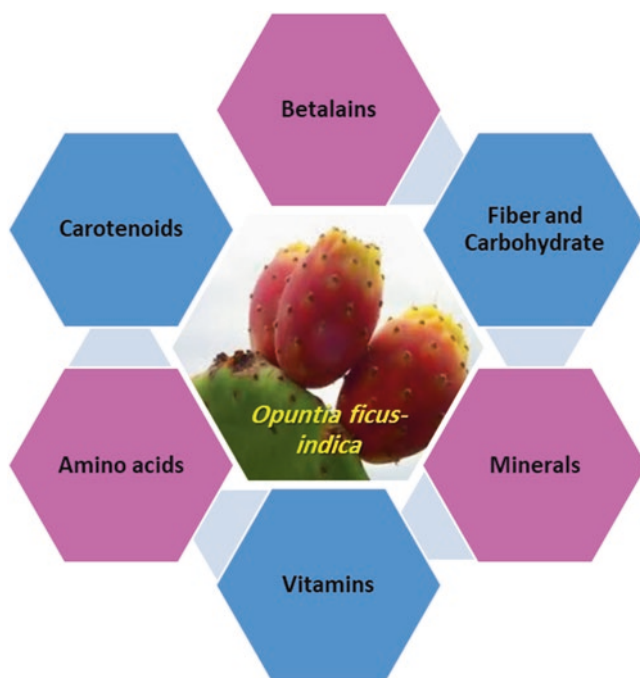
Recently, consumers and health professionals, are aware of food ingredients and their health-promoting effects (Ramadan, 2019, 2021). There is an increasing interest in the nutritional potential and active phytochemicals of food and their impacts on disease prevention. Besides, the reduction of by-products discarded from food processing became one of the European Commission's main goals (European Commission, 2019; Silva et al., 2021). According to the principles of green chemistry, it is essential to valorize bio-wastes using green methods and technologies that allow extracting active compounds that could be used to develop novel food and functional products (Stintzing et al., 2001; Anastas & Eghbali, 2010; Patel, 2012; Bouazizi et al., 2020; Oniszczuk et al., 2020; Silva et al., 2021).

*Opuntia ficus-indica* (L.) Mill. is gaining tremendous interest worldwide because it grows under difficult cultivation conditions (Feugang et al., 2006; Stintzing et al., 2001; Patel, 2012; Melgar et al., 2017; Amaya-Cruz et al., 2019; Barba et al., 2020; Silva et al., 2021; Valero-Galvan et al., 2021). *Opuntia ficus-indica* is a multi-purpose plant, not only to provide food and animal feed but also as a rich raw material of active constituents with enhancing health traits. *O. ficus-indica* fruit consists of pulp, seeds, and peel with weight levels of 25–60%, 2–10%, and 35–70%, respectively, wherein *O. ficus-indica* fruit weight average is between 45 and 250 g (Jimenez-Aguilar et al., 2014, 2015; Amaya-Cruz et al., 2019). *O. ficus-indica* minerals, dietary fibers, sugar, pigments, amino acids, vitamins, and antioxidant-rich fruits and fruit bio-wastes are considered as a rich source of functional food items and nutraceuticals (Patel, 2012; Melgar et al., 2017; Andreu et al., 2018; Du Toit et al., 2019; Silva et al., 2021). The levels of *O. ficus-indica* active components show variability due to genetic diversity and different environmental conditions (Jimenez-Aguilar et al., 2014; Amaya-Cruz et al., 2019; Barba et al., 2020).

The current chapter provides an overview of the nutritional profile and active ingredients of *O. ficus-indica* fruit, fruit by-products (peel and seeds), and cladode. Besides, this work exploits the potential of *O. ficus-indica* and its active constituents to define its possible applications in the development of pharmaceuticals, cosmetics, and novel food.

## 2 *Opuntia ficus-indica* Nutritional Composition, Bioactive Ingredients and Phyto-Constituents

The composition of different parts of *Opuntia ficus-indica* depends on several factors, including species, cultivar, climatic conditions, fertilization, maturity status, and postharvest treatment (Stintzing et al., 2001, 2005; Patel, 2012; Jimenez-Aguilar et al., 2014; Barba et al., 2017, 2020; Silva et al., 2021). Different parts of the *Opuntia ficus-indica* plant have various ingredients and nutrients (Fig. 12.1), including vitamins, minerals, amino acids, carotenoids, phenolics, betalains, and fibers.



**Fig. 12.1** Bioactive ingredients and phyto-constituents in *Opuntia ficus-indica* (L.) Mill

**Table 12.1** Nutritional composition (g/100 g) of *O. ficus-indica* different parts

Component	<i>Opuntia ficus-indica</i> part			
	Pulp	Seed	Peel	Cladode
Moisture	87–94.4	18.0	90.3	94.0
Ash	0.24–4.03	10.37	0.29	1.08
Protein	0.08–1.03	3.67	0.14	0.30
Crude protein		4.78		
Lipids	0.04–0.97	3.00–16.3	0.04–2.43	0.37–1.83
Crude lipids	0.40	5.00		
Total fiber	0.43–5.37	54.2 <sup>DM</sup>	0.65	2.7
Crude fiber	1.37–4.28	12.47	0.96	5.97 <sup>DM</sup>
Carbohydrates	92.5			5.63
Starch	4.55 <sup>DM</sup>	5.35 <sup>DM</sup>	7.12 <sup>DM</sup>	0.71 <sup>DM</sup>

Source: Silva et al. (2021)

DM dry matter

The nutritional profile and chemical composition of *O. ficus-indica* different parts is given in Table 12.1.

Moisture levels in *Opuntia ficus-indica* fruit pulp are between 80% and 90%. *Opuntia ficus-indica* has a high moisture content (88 g/100 g) and lipids content (0.50 g/100 g) (Silva et al., 2021). *Opuntia ficus-indica* pulp has lower fiber and



protein levels than the seeds and peel. Besides, *Opuntia ficus-indica* peel had greater ash levels than the seeds and pulp. *Opuntia ficus-indica* seeds contain the highest amounts of dietary fiber, protein, and lipids, with 54%, 11.8%, and 6.8% (dry base, db), respectively (Jimenez-Aguilar et al., 2014). The *Opuntia ficus-indica* pulp is characterized by high moisture and sugar levels (Silva et al., 2021). A comparative investigation (Medina et al., 2007) of the composition of *Opuntia ficus-indica* and *Opuntia dillenii* pulp showed that *O. ficus-indica* had a greater protein level (0.9 g/100 g) than *O. dillenii* (0.5 g/100 g). The pulp composition of different cultivars of *O. ficus-indica* was studied, wherein the Ait Baamrane cultivar had higher levels of protein and sugar than the Alkalaa cultivar (Dehbi et al., 2014). Besides, Salim et al. (2009) analyzed *O. ficus-indica* peel, pulp, and seeds and reported greater contents of protein and lipids in the seeds (3.6 and 3.0 g/100 g, respectively) than in the peel (0.14 and 0.10 g/100 g, respectively).

### 3 Fiber

*O. ficus-indica* by-products (i.e., peel and seeds) might be used as an essential source of fiber for human consumption (Table 12.1). Soluble and insoluble fiber might be obtained from *O. ficus-indica* peel, and the insoluble fiber from *O. ficus-indica* seeds (Jimenez-Aguilar et al., 2014). The mucilaginous components in *O. ficus-indica* fruits are related to pectic constituents (Stintzing et al., 2001). *O. ficus-indica* fruit pulp is an essential source of pectin (ca. 70% total raw fiber), wherein the peel and seeds contain cellulose at levels of 71.0% and 83.0%, respectively. The raw fiber content in *O. ficus-indica* fruit pulp is 20 g/100 g db. Cellulose, hemicellulose, pectin, and lignin were the main fiber components (Jimenez-Aguilar et al., 2014; Missaoui et al., 2020; Dick et al., 2020).

Regarding *O. ficus-indica* by-products, seeds are a good fiber source, showing higher levels than the fruit peel (El Kossori et al., 1998; Salim et al., 2009; Jimenez-Aguilar et al., 2014; Silva et al., 2021). *O. ficus-indica* pulp fiber is rich in pectin, but the peel and seeds contain high levels of cellulose (El Kossori et al., 1998; Patel, 2012). Meanwhile, *O. ficus-indica* peel pectin is characterized by the presence of galacturonic acid (64.0%), a high degree of acetylation (10.0%), a low degree of methoxylation (10.0%), and a neutral sugar content (51.0%) of which 34.5% was rhamnose and galactose (Forni et al., 1994).

### 4 Carbohydrate

Glucose and fructose are the main monosaccharides in *O. ficus-indica* pulp, while glucose is the fruit peel's principal monosaccharide. The level of those monosaccharides is responsible for the sweet flavor of *O. ficus-indica* pulp and the soluble solids level of 12–17° Brix (Jimenez-Aguilar et al., 2014; Barba et al., 2020). El

Kossori et al. (1998) studied *O. ficus-indica* chemical composition and revealed ethanol-soluble carbohydrate to be the peel and pulp's main components. In *O. ficus-indica* juices, total soluble solids recorded 127°Bx-177°Bx, with fructose and glucose being the main carbohydrates. Due to the high invertase activities, sucrose converted to readily absorbable sugar, and thus sucrose is a minor constituent in the fruit pulp (Stintzing et al., 2001).

## 5 Minerals

The mineral levels in the *O. ficus-indica* different parts are given in Table 12.2. *O. ficus-indica* pulp is considered a valuable source of minerals, including potassium, sodium, calcium, and magnesium (Jimenez-Aguilar et al., 2014). Medina et al. (2007) mentioned that magnesium and potassium levels were similar in *O. ficus-indica* orange pulp and green pulp. *O. ficus-indica* seeds are rich in minerals (i.e., phosphorus and potassium), while magnesium, calcium, and sodium were also recorded (El Kossori et al., 1998; Stintzing et al., 2001; Özcan & Al Juhaimi, 2011; Jimenez-Aguilar et al., 2014; Silva et al., 2021). High calcium (ca. 59.0 mg/100 g) and magnesium (ca. 98.4 mg/100 g) levels make *O. ficus-indica* juice effective in the prevention of cramps and osteoporosis. According to Missaoui et al. (2020), *O. ficus-indica* cladode contains high amounts of calcium (7517 mg/100 g), potassium (1684 mg/100 g), sodium (1918 mg/100 g), and magnesium (1380 mg/100 g).

Recommended Daily Allowance (RDA) of calcium, potassium, and magnesium are 1000, 2000, and 400–420 mg, respectively, in adults (Mahan & Escott-Stump, 2001). It was mentioned that 250 g of *O. ficus-indica* pulp might contribute to 140, 40, and 19 mg of potassium, calcium, and magnesium, levels that are close to 10%

**Table 12.2** Mineral levels (mg/100 g) in different parts of *O. ficus-indica*

Component	<i>Opuntia ficus-indica</i> part			
	Pulp	Seed	Peel	Cladode
Magnesium	1.05–25.0	8.07	1.47	94.1
Sodium	0.06–1.29	0.44	0.11	1.71
Potassium	11.1–158	64.4	9.48	224
Calcium	0.69–40.9	17.3	1.52	177
Manganese	0.10–4.89	<0.83	0.13	0.78
Iron	0.20–3.35	12.1	0.47	0.13
Zinc	0.07–1.63	4.16	0.13	0.37
Copper	0.001–0.14	<0.83	0.19	0.06
Phosphorus	0.006–0.26	162	0.53	0.09
Molybdenum	<0.31 <sup>DM</sup>	<0.33 <sup>DM</sup>	<0.34 <sup>DM</sup>	

Source: Silva et al. (2021)

DM dry matter

of RDA (Jimenez-Aguilar et al., 2014). Magnesium, calcium, and potassium are also applied in sports and energy drinks to uphold the mineral pool during physical exercise (Stintzing et al., 2001).

## 6 Vitamins

Vitamins are nutritionally essential compounds of the *O. ficus-indica* (Table 12.3). The levels of vitamins found in *O. ficus-indica* vary among the plant parts. *O. ficus-indica* pulp is a rich source of ascorbic acid (vitamin C), wherein its amount ranged from 17.0 to 46.0 mg/100 g (Galati et al., 2003; Stintzing et al., 2005; Jimenez-Aguilar et al., 2014; Silva et al., 2021). RDA value of vitamin C is 60 mg for adult women and men, and of 45 mg for children (Mahan & Escott-Stump, 2001). Consumption of 250 mL of *O. ficus-indica* juice might be enough to supply about 25% of adults' requirements (Jimenez-Aguilar et al., 2014).

*O. ficus-indica* peel is a good source of vitamin E, especially  $\alpha$ -tocopherol (1761 mg/100 g total lipid) (Ramadan & Mörsel, 2003a, b, c; Jimenez-Aguilar et al., 2014; Silva et al., 2021). In *O. ficus-indica* pulp,  $\alpha$ -tocopherol is found in greater levels, compared with other vitamin E forms. Ramadan and Mörsel (2003a, b) compared *O. ficus-indica* seed oil with the pulp oil and reported that the pulp oil had a higher level of vitamin E, especially  $\delta$ -tocopherol (442 mg/100 g total lipid). Besides, *O. ficus-indica* seed oil had a higher level of  $\gamma$ -tocopherol (33 mg/100 g total lipid).

**Table 12.3** Vitamins levels in the different parts and products of *O. ficus-indica*

Component	Unit	Pulp	Pulp oil	Seed oil	Peel	Cladode
Vitamin C	mg/100 g	5.17–33.0				
	mg ascorbic acid eq/100 g				109.7	
Ascorbic acid	mg/100 g	17.2–29.0			59.8	1.83
Total vitamin E	mg/100 g		527	40.3		
	mg/100 g of total lipids				2180	
$\alpha$ -Tocopherol	mg/100 g		84.9	5.60		
	mg/100 g of total lipids				1760	
$\beta$ -Tocopherol	mg/100 g		12.6	1.20		
	mg/100 g of total lipids				222	
$\delta$ -Tocopherol	mg/100 g		422	0.50		
	mg/100 g of total lipids				26	
$\gamma$ -Tocopherol	mg/100 g		7.90	33.0		
	mg/100 g of total lipids				174	
Vitamin K	mg/100 g		53.2	525		
	mg/100 g of total lipids				109	

Sources: Silva et al. (2021), Ramadan and Mörsel (2003a, b)

Vitamin K is a cofactor in the synthesis of the coagulation inhibitor, blood coagulation factors, and the proteins of the bone matrix. RDA values for vitamin K are 80 and 65 $\mu$ g for adult men and women, respectively (Mahan & Escott-Stump, 2001). *O. ficus-indica* seed and pulp oils contain high levels of vitamin K (Table 12.3), wherein 150 $\mu$ g of *O. ficus-indica* oils could meet the requirements of vitamin K (Ramadan & Mörsel, 2003a; Jimenez-Aguilar et al., 2014).

## 7 Amino Acids

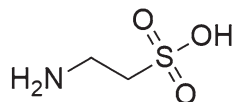
Protein level was found to be high in *O. ficus-indica* seeds (El Kossori et al., 1998). The primary amino acids (Table 12.4) found in the *O. ficus-indica* pulp are serine and proline, and  $\gamma$ -aminobutyric acid (Ali et al., 2014; Stintzing et al., 1999, 2001; Jimenez-Aguilar et al., 2014). Free amino acids in *O. ficus-indica* comprised all essential amino acids (Stintzing et al., 2001). Meanwhile, *O. ficus-indica* pulp comprises various free amino acids, predominating glutamine, proline, and taurine (Jimenez-Aguilar et al., 2014).

**Table 12.4** Amino acid levels in the different parts of *O. ficus-indica*

Component	Pulp		Seed meal	Cladode
	$\mu$ mol/L	mg/L	g amino acid/100 g protein	g/100 g protein
Reference	Ali et al. (2014)	Stintzing et al. (1999)	Sawaya et al. (1983)	Hernández-Urbiola et al. (2010)
Taurine		323.6		
Aspartate	844		8.60	0.61
Threonine	120	13.1	3.96	1.38
Serine	967	175	4.14	0.48
Asparagine	253	41.6		
Glutamine	1583	346		
Proline	6461	1265	5.66	0.45
Glycine	174	11.3	7.67	0.36
Alanine	353	8.72	4.58	0.46
Citrulline	79.1	16.2		
Valine	254	39.3	5.69	0.58
Methionine	189	55.2	2.61	0.15
Isoleucine	241	31.1	3.66	0.67
Leucine	218	20.6	6.90	0.76
Tyrosine	208	12.3	3.56	0.21
Phenylalanine	337	23.3	4.46	1.37
Histidine	505	45.2	2.46	0.15
Tryptophan	117	12.6	0.90	0.16
Arginine	375	30.5	14.6	0.16

Source: Silva et al. (2021)

Fig. 12.2 Taurine structure

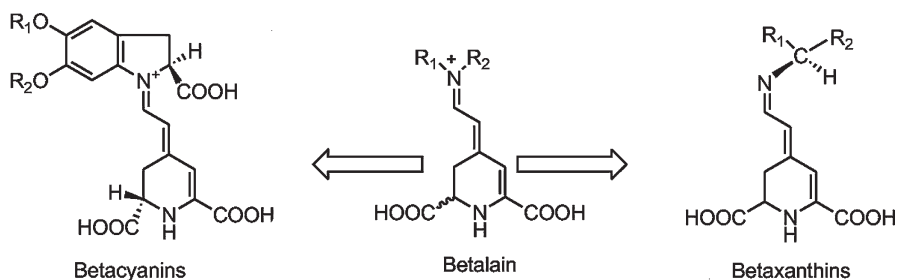


Concerning the amino acids content in *O. ficus-indica* seeds, the primary amino acids are glutamic acid (20 g/100 g protein) and arginine (14 g/100 g protein) (Sawaya et al., 1983). Stintzing et al. (1999) studied the amino acids present in pulps of different *O. ficus-indica* cultivars and mentioned that taurine (semi-essential amino acid), proline, and glutamine were the primary acids (Stintzing et al., 2001, 2005; Silva et al., 2021). Taurine (Fig. 12.2) is considered a cellular protective amino acid. In addition, taurine is a preventive agent of hepatic steatosis or fatty-liver disease and considered a stabilizer of the cell membrane, providing neuro-modulation, osmoregulation, and hypercholesterolemia (Jimenez-Aguilar et al., 2014; Chang et al., 2010). In addition, taurine is involved in modulating the inflammatory response and showed antioxidant potential (Devamanoharan et al., 1998; Wu et al., 1999; Stintzing et al., 2005; Silva et al., 2021). Besides, taurine is commonly added to energy and sports drinks (Stintzing et al., 2001, 2005).

## 8 Carotenoids

Total carotenoids content was reported in the whole *O. ficus-indica* as 2.6–2.9 $\mu$ g  $\beta$ -carotene equivalents ( $\mu$ g  $\beta$ CE)/g (Jimenez-Aguilar et al., 2014). As reported (Tesoriere et al., 2005; Jimenez-Aguilar et al., 2014), *O. ficus-indica* contains 0.015, 0.014, and 0.045 $\mu$ g  $\beta$ CE/g in the pulp of yellow, white, and red species, distributed as *trans*- $\beta$ -carotene (80–87%),  $\alpha$ -carotene (*ca.* 5.0%), *trans*-lycopene (8.0–12.0%) and phytofluene (0.90–1.40%).

In *O. ficus-indica* cladodes, lutein (102–187 $\mu$ g/100 g db),  $\beta$ -carotene (82–119 $\mu$ g/100 g db), and  $\beta$ -cryptoxanthin (45–72 $\mu$ g/100 g db) were quantified (Jaramillo-Flores et al., 2003). As reported by Cano et al. (2017), *O. ficus-indica* peel had higher carotenoids than in the pulp. The same authors (Cano et al., 2017) reported that the major carotenoids in *O. ficus-indica* peel of Verdal (orange) variety and Sanguinos (red) variety, respectively, are lutein (765 and 1130 $\mu$ g/100 g),  $\beta$ -carotene (170 and 200 $\mu$ g/100 g) and violaxanthin (87.6 and 93.6 $\mu$ g/100 g). In addition, lycopene was detected in *O. ficus-indica* peel (45.6 $\mu$ g/100 g), whereas only trace amounts were detected in the pulp. In *O. ficus-indica* pulp of the varieties mentioned above, the predominant carotenoids were lutein (203 and 201 $\mu$ g/100 g),  $\beta$ -carotene (79.0 and 37.0 $\mu$ g/100 g), violaxanthin (3.1–5.7 $\mu$ g/100 g), and zeaxanthin (12.0 and 14.0 $\mu$ g/100 g) (Cano et al., 2017; Silva et al., 2021).



**Fig. 12.3** Structure of betalain, betaxanthins and betacyanins

## 9 Betalains

Betalains, aromatic compounds derived from tyrosine, are vacuolar pigments contain a nitrogenous core structure (betalamic acid). The presence of betalamic acid was confirmed in *O. ficus-indica* fruits (Stintzing et al., 2001). Betalain compounds include two classes (Fig. 12.3): betaxanthins (yellow) and betacyanins (red-violet), and their levels vary upon fruit color (Jimenez-Aguilar et al., 2014; Cano et al., 2017; Kanner et al., 2001; Slimen et al., 2016). Meanwhile, betalains are effective radical scavengers and act as antioxidants in biological systems (Stintzing et al., 2001; Jimenez-Aguilar et al., 2014; Cano et al., 2017; Kanner et al., 2001; Slimen et al., 2016). Stintzing et al. (2005) reported the levels of betaxanthins (32.7–553 mg/kg) and betacyanins (586–10.5 mg/kg) in different *O. ficus-indica* cultivars. As reported by Cano et al. (2017), *O. ficus-indica* peel contains high values of betacyanins (1.1 and 2.5 mg betanin/100 g) and betaxanthins (1.7 and 2.0 mg indicaxanthin/100 g) for *O. ficus-indica* Verdal and Sanguinos varieties, respectively (Jimenez-Aguilar et al., 2014; Silva et al., 2021).

## 10 Conclusions

The trend towards functional ingredients that are promoting well-being and health is increased. *O. ficus-indica* as a multi-ingredient fruit that holds a promising answer for tailor-made novel food and nutraceuticals by embracing functional compounds such as betalains, carotenoids, readily absorbable carbohydrates, taurine, magnesium, calcium, vitamin C, and soluble fibers. *O. ficus-indica* bioactives exhibited diverse health-promoting effects, including hepatoprotective, immunomodulatory, antioxidant, anti-atherogenic, anticarcinogenic, anti-ulcerogenic, and hepatoprotective traits.

Considering the nutritional composition of *O. ficus-indica*, it could be said that *O. ficus-indica* fruit, fruit by-products (skin and seeds), and cladode could be industrially exploited. There is a potential of *O. ficus-indica* fruit, fruit by-products (peel

and seeds), and cladode to develop novel food and to extract added-value phyto-extracts that could be applied in cosmetic, food, nutraceutical and pharmaceutical products. The transformation of *O. ficus-indica* by-products (peel and seeds) and cladode into novel raw materials make it possible to move to a closed economic systems. It is essential to continue investing in new bioactives with attractive biological traits and to optimize their extraction techniques, making them environmentally friendly. Besides, it is anticipated to study the mechanisms of action of *O. ficus-indica* phytochemicals regarding its health impacts.

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# Chapter 13

## *Opuntia* Fiber and Its Health-Related Beneficial Properties



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**Abstract** Both the nopal cactus (*Opuntia* spp.) and its fruit (prickly pear) have an interesting nutritional composition since it has been reported that they are rich in phenolic compounds, sugars, betalains, ascorbic acid, and fiber. These compounds are related to functional properties of interest to human health. Fiber is associated with the prevention of various health problems related to the modern lifestyle, such as diabetes, cardiovascular problems, and obesity. The fiber in nopal green leaves (paddles) is both soluble and insoluble, helping lower blood glucose and cholesterol levels. This chapter reviews the current information on the main species consumed of the *Opuntia* genus, the different types of fiber that they contain, their chemical composition, the degradation of fiber in the digestive tract, the consumption of nopal fiber, and its effect on diabetes, cardiovascular problems, obesity among other health complications, as well as different technological applications of fiber

**Keywords** Cactaceae · Health-benefits · Mucilage · *Opuntia* fiber · *O. ficus-indica*

### Abbreviations

AACC	American Association of Cereal Chemists
AGCC	Short chain fatty acids
ASCVD	Atherosclerotic cardiovascular disease
DF	Dietary fiber
FAO	Food and Agriculture Organization of the United Nations
FSA	European Food Safety Authority

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IDF	Insoluble dietary fiber
SDF	Soluble dietary fiber
WHO	World Health Organization

## 1 Introduction

*Opuntia* is a dicotyledonous angiosperm plant. This genus belongs to the Cactaceae family (El-Mostafa et al., 2014). It is taxonomically diverse. More than 200 species belonging to this genus have been reported worldwide; 114 occurred in Mexico (Vigueras & Portillo, 2001). These species domesticated one *O. ficus-indica* (Astello-García et al., 2015). Different *Opuntia* species have been used for foodstuff and fodder by people in Mexico and the Southern United States of America for hundreds of years (González-Stuart, 2013). Currently, the *Opuntia* genus has become an important crop worldwide. However, it is considered a noxious plant (Astello-García et al., 2015). *Opuntia* invasions are considered a major threat to conserving native species and ecosystems (Vila et al., 2003). However, this plant is underutilized around the world. Aparicio-Fernández et al. (2017) indicated that only 4 out of 29 *Opuntia* species identified in Jalisco State, Mexico are used for vegetable or fruit (*Opuntia ficus-indica*, *O. streptacantha*, *O. undulata*, and *O. megacantha*) production, and only 3 species (*O. microdasys*, *O. pumila*, and *O. pubescens*) are employed with ornamental purposes. Based on these antecedents, this chapter's objectives are to discuss the updated information on the main edible *Opuntia* species, its fiber types and degradation, their chemical composition, and its effect on different health problems.

### 1.1 Origin and Distribution

Mexico is recognized as the center of *Opuntia*, wherein most of the 114 species identified in this country grow wild (Astello-García et al., 2015). In Spanish, the plants of this genus are known as “nopales” and this word came from the Náhuatl or Aztec word “Nopale” (González-Stuart, 2013). These plants can be found mainly in underutilized arid and desert areas (Mayer & Cushman, 2019), where, because of the hostile environment, few crops can be cultivated (Vigueras & Portillo, 2001). However, this plant plasticity allows them to adapt to other environments. El-Mostafa et al. (2014) consignee that *Opuntia* is also adapted to tropical and subtropical regions, especially those with arid and semi-arid weather. Currently, because of their growth rate and low-input requirements (Mayer & Cushman, 2019), *Opuntia* plants are mainly distributed in America, Africa, and the Mediterranean basin (Santos-Díaz et al., 2017). However, in some countries, this plant is considered a big problem for conserving native plant species. This problem is exceptionally high in

some areas such as shrublands and woodlands located near urban areas, agricultural abandonment regions, and those with afforestation (Vila et al., 2003). Some *Opuntia* species adapt better to specific environments. Vila et al. (2003) reported that *O. maxima* were found more frequently in the shrublands, while *O. stricta* was more represented in the woodlands that were former crops.

## 1.2 Outstanding *Opuntia* Species

Azizi-Gannouni et al. (2020) performed the taxonomical analyses of 45 ecotypes of *Opuntia* species collected from 9 different regions of the world and identified 5 main clusters. In the same study, it was found that *Opuntia ficus-indica* (Pr28) formed a separate cluster and displayed a distinct branching pattern, and its ancestor is still unclear. *O. ficus-indica* differs from the proposed taxonomy, and it seems to fall far outside of the current domestication gradient (Astello-García et al., 2015). Varietal differences in chemical composition (crude fiber, lipid, carbohydrates, ash, ascorbic acid, protein, and carotenes) have been reported among *O. amyclaea*, *O. inermis*, and *O. ficus-indica* (Hegwood, 1990). Some *Opuntia* species have specific features, e.g., *Opuntia undulata* contained a high amount of phenolics [905  $\mu\text{g}$  of gallic acid equivalents (GAE)/g] (Santos-Zea et al., 2011), *O. inermis* contains high levels of crude protein and phosphorus (Hegwood, 1990), *Opuntia robusta* var. *Gavia* has a high antioxidant capacity [738  $\mu\text{mol}$  of Trolox equivalents (TE)/g] (Santos-Zea et al., 2011), while *Opuntia maxima* and *O. stricta* have a high competitive capacity (Vila et al., 2003). Some *Opuntia* species have been identified as a potential source of food and economic incomes for people living in arid regions. Some of these species are *O. robusta*, *O. cantabrigiensis*, *O. rastrera*, *O. lindheimeri*, *O. leptocaulis*, *O. leucotricha*, and *O. streptacantha* (Aparicio-Fernández et al., 2017).

## 1.3 Chemical Composition

Santos-Díaz et al. (2017) mentioned that all *Opuntia* parts (pear, roots, cladodes, seeds, and juice) have beneficial properties for human health because of their high phenolic acid content, antioxidants, and pigments. Besides, the *Opuntia* plant is rich in vitamins, polyunsaturated fatty acids (PUFA), and amino acids (El-Mostafa et al., 2014), steroids, sugar, minerals, and fibers (Kumar & Kumar-Sharma, 2020), biopeptides, and soluble fibers (Santos-Díaz et al., 2017). During the evening malic and citric acids contents increase because of the crassulacean type acid metabolism of *Opuntia* (Hegwood, 1990). Other chemical compounds reported in *Opuntia* tissue are pigments (carotenoids, and betalains) (Santos-Díaz et al., 2017), and amino acids (methionine, leucine, and cysteine) (Hegwood, 1990).

The major phenolic compounds found in *Opuntia* plants are kaempferol 3-*O*-robinobioside-7-*O*-arabinofuranoside, isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside), eucomic acid, and isorhamnetin 3-*O*-galactoside (Astello-García et al., 2015). These phenolic compounds confer a high antioxidant activity to *Opuntia* tissue, comparable to cranberries and blackberries (Santos-Zea et al., 2011). Besides, other phenolic compounds are found in small quantities and only in a specific *Opuntia* species group. Astello-García et al. (2015) indicated that kaempferol 3-*O*-arabinofuranoside was found only in wild species, while quercetin 3-*O*-rhamnosyl-(1 → 2)-[rhamnosyl-(1 → 6)]-glucoside was detected only in the tissue of domesticated species. Another compound with antioxidant capacity that has been reported in *Opuntia* tissue is ascorbate (Santos-Díaz et al., 2017).

*Opuntia* crude protein content can be affected by environmental conditions. Mayer and Cushman (2019) reported a crude protein content of 71 g/kg of dry mass when *Opuntia* plants were grown under field conditions, while when this plant was grown under greenhouse conditions, the crude protein content increased to 264 g/kg. Other environmental conditions that may affect the nutritional composition of *Opuntia ficus-indica* (L.) are age and season (Chiteva & Wairagu, 2013). Growing conditions affect the contents of some bromatological parameters such as acid and neutral detergent fiber (cellulose, hemicellulose, and lignin) content, which were higher when plants were grown under field conditions than when plants were grown under greenhouse conditions (Mayer & Cushman, 2019). Hegwood (1990) consignee that when titratable acidity and total carbohydrates increase, protein and crude fiber decrease.

## 1.4 Potential Applications

**Food.** González-Stuart (2013) consignee that different cactus species have been used for nourishment for hundreds of years. The most domesticated specie is *O. ficus-indica*, which is used as a nutritional compound in various dietary and value-added products. Cactus is an excellent natural reservoir of energy (Chiteva & Wairagu, 2013), and both fruits and stems can be consumed (Aparicio-Fernández et al., 2017). Besides, *Opuntia* nutritional components and antioxidants can be added as a food supplement (Chiteva & Wairagu, 2013). *Opuntia* cladodes are relatively low in protein. Hegwood (1990) mentioned that nopal protein has a biological value of 72.6 relative to egg protein, and this protein is higher in younger cladodes than older. In comparison to other vegetables, *Opuntia* cladodes juice has, on average, a pH of 4.6, moderate amounts of vitamin C and vitamin A, titratable acidity of 0.45%, and soluble solids 6.9% (Hegwood, 1990). The same author indicated that in 100 g of nopal tissue, there are 0.1 g of protein, 1.1 g of crude fiber, 1.3 g of ash, 0.1 g of lipids, 0.82 g of simple sugar, 4.6 g of complex carbohydrates, 28.9 µg of carotenes, and 12.7 mg of ascorbic acid.

**Traditional medicinal uses.** Cactus (*Opuntia* spp.) has been used in traditional folk medicine for hundreds of years (González-Stuart, 2013), especially for the

treatment of chronic diseases (diabetes, obesity, cardiovascular diseases, and cancer) (Santos-Díaz et al., 2017), wound healing, and urinary tract infection (Kumar & Kumar-Sharma, 2020). The biological activities (inflammatory, antioxidant, hypoglycemic, antimicrobial, and neuroprotective properties) are due to the chemical compounds that *Opuntia* tissue possesses (El-Mostafa et al., 2014). The species more used for disease treatments in traditional medicine are *Opuntia ficus-indica* and *Opuntia streptacantha* (Aparicio-Fernández et al., 2017).

**Therapeutic uses.** Recently, scientific information has been developed on the potential of *Opuntia* species as a treatment for different diseases because the pharmaceutical agents of these plants, which may be used to develop value-added products (Santos-Díaz et al., 2017). Kumar and Kumar-Sharma (2020) revised the chemistry, pharmacognosy, pharmacology, and bio-applications of *Opuntia* species. They reported different biological activities such as anti-diabetic, antiviral, neuroprotective, anticancer, hepatoprotective, anti-inflammatory, antibacterial, alcohol hangover, antiulcer, and antioxidant, which could provide a protective effect of *Opuntia*-enriched diets against type 2 diabetes (González-Stuart, 2013) and other chronic diseases (Santos-Díaz et al., 2017). The medicinal properties of *Opuntia* spp. are attributed to its phytochemicals and its biopeptides and soluble fibers (Santos-Díaz et al., 2017).

**Other uses.** Besides to be a potential source of food and medicine, *Opuntia* plants offer other benefits, which may increase the economic incomes of the people living in semi-arid and arid areas (Aparicio-Fernández et al., 2017). *Opuntia* plants can be used for beverage elaboration, source of dyes (Kumar & Kumar-Sharma, 2020), in cosmetics, and as natural fences (Vigueras & Portillo, 2001), soil conservation purposes (Azizi-Gannouni et al., 2020), soil erosion prevention, and wastewater decontamination (Kumar & Kumar-Sharma, 2020), candy elaboration, construction and housing protection (Aparicio-Fernández et al., 2017). In semi-arid and arid regions, where water availability is restricted, production of crops typically used for livestock fodder or forage is either uneconomical or unsustainable (Mayer & Cushman, 2019). In this case, an economical option as fodder for cattle is cactus (Vigueras & Portillo, 2001).

## 2 Distribution and Consumption of *Opuntia* spp. in Mexico

Since pre-Hispanic times, the *Opuntia* genus has been used for various purposes such as animal fodder, arts, traditional medicine, and human consumption (Ochoa & Barbera, 2018; Torres-Ponce et al., 2015). In this sense, the fruits called “tunas” or “xoconostles” are currently consumed, as well as the tender cladodes called “nopal” or “nopalitos” (Monroy-Gutiérrez et al., 2017; Ochoa & Barbera, 2018). There is a great variety of species of the genus *Opuntia* spp. However, the species with the highest economic value worldwide is *Opuntia ficus-indica* (De Luna-Valadez et al., 2016). Regarding America, the species *O. ficus-indica* is found from Canada to Patagonia, highlighting Brazil with an area of around 500,000 ha, Peru

with 10,000 ha, Chile with 934.4 ha, and Argentina with 1650 ha. These countries have a wide distribution of *Opuntia*, managing cactus plants and trading of their fruits for consumption, as well as, fodder use (Dubeux et al., 2018; Ochoa & Barbera, 2018).

Meanwhile, Mexico is the center of genetic origin and distribution of the *Opuntia* genus (Esquivel, 2004; Figueroa-Cares et al., 2010), the reason why Mexico is the world's largest producer of both nopal vegetable and fruit (prickly pear or xoconostle) (Scheinvar et al., 2015). The *Opuntia* genus inhabits wild, mainly in arid and semi-arid areas of the geographical territory of Mexico (Espinoza-Sánchez et al., 2014; Scheinvar et al., 2015). The main cultivated species are *O. megacantha*, *O. ficus-indica*, *O. xoconostle*, *O. streptacantha*, *O. leucotricha*, *O. robusta*, *O. chaveña* and *O. hyptiacantha* (Ochoa & Barbera, 2018). There is a wide diversity of species of the *Opuntia* genus (Márquez-Berber et al., 2012), which cover approximately 3,000,000 ha throughout Mexico. The states of Aguascalientes, Mexico City, Baja California, Morelos, Mexico, Michoacan, Jalisco, Puebla, Tamaulipas, and Zacatecas are the largest commercial producers of *Opuntia* spp. with a planted area of 11,594.80 ha, which generates a production volume of 824,140.38 tons of nopalitos (young cladodes) (Table 13.1), while the rest of the states cover a planted area of 1258.64 ha, generating in 2018 a production volume of 29,354. Eighty-five tons of nopalitos, according to SIAP (Table 13.2). Thousands of families feed on the *Opuntia* national production (Ochoa & Barbera, 2018; SADER & SIAP, 2019), with an annual per capita consumption of 6.4 kg (SADER & SIAP, 2019), since nopalitos represent the food and economic sustenance of Mexican families (Márquez-Berber et al., 2012; Ávalos et al., 2013).

Concerning prickly pear production, there is a planted area of approximately 46,555.91 ha with an annual production of 471,637.78 tons, bearing in mind that the main states producing prickly pear are Hidalgo, Puebla, Guanajuato, San Luis Potosí, Mexico, and Zacatecas; these last two states produced in 2018, the highest volume of prickly pear production in Mexico (Table 13.3) (SAGARPA & SIAP,

**Table 13.1** Main nopalitos (*Opuntia* spp.) producing states nationwide in Mexico

No.	Main producing states	Sown (ha)	Production volume (ton)	References
1	Aguascalientes	186.04	10,459.43	SADER and SIAP (2019) SAGARPA and SIAP (2018)
2	Baja California	403.95	14,140.13	
3	Ciudad de México	2682.00	203,888.00	
4	Jalisco	734.50	35,231.35	
5	Mexico	965.09	86,671.83	
6	Michoacán	741.80	21,724.15	
7	Morelos	4141.00	396,874.20	
8	Puebla	428.00	31,924.05	
9	Tamaulipas	957.42	11,976.49	
10	Zacatecas	355.00	11,250.75	
	Total	11,594.80	824,140.38	

**Table 13.2** Nopalitos (*Opuntia* spp.) producing states nationwide in Mexico

Other producing states	Sown (ha)	Production volume (ton)	Reference
Baja California Sur	86.00	2902.00	SADER and SIAP (2019) SAGARPA and SIAP (2018)
Coahuila	2.00	92.60	
Colima	14.50	315.41	
Durango	60.10	2772.49	
Guanajuato	247.50	6228.95	
Guerrero	11.00	188.11	
Hidalgo	89.40	6861.65	
Nayarit	54.94	214.22	
Nuevo Leon	4.20	159.60	
Oaxaca	147.50	1690.19	
Queretaro	51.00	1445.40	
San Luis Potosi	300.00	2492.33	
Sinaloa	1.00	7.80	
Sonora	149.00	3093.80	
Tlaxcala	8.00	100.30	
Veracruz	30.50	740.00	
Yucatán	2.00	50.00	
Total	1258.64	29,354.85	

**Table 13.3** Prickly pear producing states in Mexico

States	Sown (ha)	Production volume (ton)	Reference
Aguascalientes	420	2875.1	SAGARPA and SIAP (2018)
Durango	4	51.14	
Guanajuato	1801.5	22,127.4	
Hidalgo	4501	20,524.2	
Jalisco	2013	12,563.88	
México	15,969.6	185,623.85	
Michoacán	3.5	12.95	
Oaxaca	103.3	441.48	
Puebla	5428.41	119,382.15	
Querétaro	322	599.8	
San Luis Potosí	3096.1	17,116.01	
Tamaulipas	915	1026	
Tlaxcala	39	201.9	
Veracruz	18	111.6	
Zacatecas	11,871.5	88,980.32	
Total	46,555.91	471,637.78	



**Table 13.4** Forage Nopal (*Opuntia* spp) producing States in Mexico

States	Sown (ha)	Production volume (ton)	Reference
Aguascalientes	829	29,792.4	SAGARPA and SIAP (2018)
Coahuila	15,280.1	119,171.39	
Jalisco	3	90.78	
Zacatecas	862.5	28,128.3	
Total	16,974.6	177,182.87	

2018; Granillo Macias et al., 2019). Additionally, production of fodder cactus in Mexico is obtained with use to feed cattle, sheep, and goats, where the main producing states of fodder cactus are Aguascalientes, Coahuila, Jalisco, and Zacatecas (Table 13.4), which generate a production volume of 177,182.87 tons of fodder cactus. The fodder cactus is included in the diets of the animals during different livestock production systems in the arid and semi-arid zones of Mexico since the fodder cactus helps to compensate the malnutrition of the animals and also improves the quality of meat and milk (Torres-Ponce et al., 2015; Dubeux et al., 2018; SADER & SIAP, 2019). It should be noted that the cultivation areas of the *Opuntia* genus are continually growing (Maki-Díaz et al., 2015).

Nopalitos are currently exported to foreign markets such as Belgium, Canada, South Korea, El Salvador, United Arab Emirates, USA, United Kingdom, Czech Republic, Switzerland, Thailand, Taiwan, and Japan. However, the USA is the main client of Mexican prickly pear, with an acquisition of 51,598.00 tons, which generates a value of 19,224,360.00 US dollars (SAGARPA & SIAP, 2018; SADER & SIAP, 2019). Exports are carried out under official Mexican quality standards (NMX-FF-068-SCFI-2006), achieving quality, acceptability, and commercial success (Maki-Díaz et al., 2015). Thus, the *Opuntia* genus is a profitable crop in Mexico, given the worldwide demand, which allows obtaining higher incomes for this commodity (SADER & SIAP, 2019).

Likewise, in the Mexican market, there is a commercialization of the nopalitos almost all the year, with an increase during February, March, April (holly weak), May, and June, while there is a decrease during January, September, October, and November (SAGARPA & SIAP, 2018; SADER & SIAP, 2019). In particular, nopalitos are used in the Mexican cuisine and the ordinary diet of Mexicans, either in salads, juice, smoothie, consumed fresh or prepared in different dishes (Valdez et al., 2008; Maki-Díaz et al., 2015; SAGARPA & SIAP, 2018; SADER & SIAP, 2019) since they are rich in vitamin C, minerals (calcium and potassium) and dietary fiber either insoluble (cellulose, hemicellulose, and lignin) or soluble (pectin, mucilage) (Sáenz et al., 2004; Valdez et al., 2008; Maki-Díaz et al., 2015; Torres-Ponce et al., 2015; Vargas-Rodríguez et al., 2016; Monroy-Gutiérrez et al., 2017). All the nutritional properties that nopalitos contain are of great interest for human health and for the food industry, where different minimally processed products such as pickles, vinegar, brines, pickles, prickly pear flour (to prepare tortillas), sauces, jams, sweets, juices, and additives (dyes, gums) are obtained. Similarly, they focus on the pharmaceutical industry because nopalitos are used to formulate food

supplements rich in fiber, nopal powder capsules, and gastric protectors based on mucilage. Indeed nopal mucilage (hydrocolloid) is rich in sugars (L-arabinose, L-rhamnose, D-xylose, and D-galactose), which have also been given other uses such as the production of edible films, water purification, making bricks and plaster, binders, and gels (Sáenz et al., 2004; Valdez et al., 2008).

On the other hand, prickly pears are also marketed in the local Mexican markets. Mexico is one of the largest prickly pear producers, achieving a wide distribution and marketing of this fruit (Granillo Macias et al., 2019). Prickly pear fruits can be of different varieties and colors (white, red, yellow, and green) according to the species to which they belong. Fruits are obtained in May (early ripening), August (intermediate ripening), and November (late ripening). Likewise, prickly pear consumption is generally fresh (ripe) or processed into jams, ice creams, syrups, preserves, juices, dehydrated products, among other products (Monroy-Gutiérrez et al., 2017). Additionally, these fruits are rich in phenolic compounds, antioxidants, betalains (yellow betaxanthines and red betacyanins), vitamins (C, and E), dietary fiber (mucilage, and pectin) (Sáenz et al., 2004; Albano et al., 2015), sugars (glucose and fructose), proteins, minerals, amino acids (proline, taurine, and serine), tocopherol, and PUFA (linoleic acid) (Nazareno, 2018).

The *Opuntia* genus has been reported as an important genetic resource with great nutritional value and functional properties (Figueroa-Cares et al., 2010); it produces cladodes, young cladodes (nopalitos), and prickly pear. Therefore, it is crucial to consider that a diet rich in this vegetal with a daily intake of approximately 25 g fruit and 25 g prickly pear (Wolfram et al., 2003; Nazareno, 2018), is of great benefit in the consumer's health, helping to prevent various diseases, thanks to the fact that the *Opuntia* genus contains bioactive compounds such as phenolics (Zou et al., 2005). Thus, it also helps reduce obesity because of hypercholesterolemia and the decrease of blood glucose, given the high content of fibers (Ennouri et al., 2006). *Opuntia* tissue also has an anti-inflammatory action because of its betalains (Allegra et al., 2005), among other medicinal properties (Nazareno, 2018). Therefore, the Food and Agriculture Organization of the United Nations (FAO) indicates that the consumption of nopalitos and prickly pear of the *Opuntia* genus should be increased (FAO, 2017). It is necessary to continue with studies on human and *Opuntia* relationships to learn more about the benefits of consuming this plant.

### 3 *Opuntia* Dietary Fiber: Definition and Types

Dietary fiber (DF) refers to plant-originated polysaccharides that cannot be digested and absorbed in the gastrointestinal tract. This term was not established from the beginning, but rather evolved and thanks to the studies of various institutions such as the American Association of Cereal Chemists (AACC) and the Institute of Medicine (IOM) of the US National Academy, FDA, FAO/WHO, CAC, Codex Alimentarius Commission (2008), and European Food Safety Authority (EFSA), to finally reach a consensus and establish the term (Li & Komarek, 2017). Likewise,

there is a classification of DF according to their structure, when the polysaccharides are categorized into linear or nonlinear molecules and according to their solubility and structure: insoluble fiber (e.g., lignin, cellulose, and hemicellulose) and soluble dietary fiber (e.g., pectin, inulin, and mucilage). The last classification is the most used and classified as total dietary fiber (Nair et al., 2010).

DF is present in several natural sources as fruits, vegetables, plants and agro-industrial residues. One of these natural sources is *Opuntia* spp. which is rich in other bioactive compounds like antioxidants, minerals, and vitamins (Santos-Díaz et al., 2017). The interest in the studio of DF of *Opuntia* spp. has been increased in the last years due to its particular characteristics founded in its fruits called prickly pear and, in the cladodes, both widely consumed in Mexico and because of their content of soluble and insoluble polysaccharides or total dietary fiber (Peña-Valdivia et al., 2012).

### 3.1 Soluble Dietary Fiber in *Opuntia* spp.

In *Opuntia* spp., soluble dietary fiber (SDF) is principally represented by mucilage and pectin (Maki-Díaz et al., 2015). Mucilage is a complex polymeric substance composed mainly of carbohydrates with highly branched structures, including L-arabinose, D-galactose, L-rhamnose, and D-xylose and galacturonic acid in different proportions, but this may vary from specie to specie (Gebresamuel & Gebre-Mariam, 2012; Monrroy et al., 2017). The mucilage structure is proposed as two distinctive water-soluble fractions: one is pectin with gelling properties with  $\text{Ca}^{2+}$ , and the other is a mucilage without gelling properties (Trachtenberg & Mayer, 1982). The principal function of mucilage in cladodes is retaining water. It is founded in mucilaginous cells, which are in the chlorenchyma and parenchyma of the epidermis of this part of the plant (Sepúlveda et al., 2007).

Pectin plays an important role in the plant defense mechanisms against plant pathogens and wounding due to their anionic nature. This component varies in composition depending on the source and conditions of extraction, location, and other environmental factors (Morales-Martínez et al., 2018). The pectin's main component is a backbone chain structure of  $\alpha$ -(1  $\rightarrow$  4)-linked D-galacturonic acid units interrupted by the insertion of (1  $\rightarrow$  2) linked L-rhamnopyranosyl residues in adjacent or alternate positions. The linear segments consisting predominantly of galacturonan are called homogalacturonans (Saffer, 2018). There are different methods to determine the carbohydrate composition and have been proved in several components of *Opuntia* spp. For example, nopal pectin sugar composition extracted by the alkaline process is uronic acid 56.3% rhamnose 0.5%, arabinose 5.6%, galactose 6.5%, and xylose 0.9%. In contrast, in acidic process, the sugar composition is uronic acid 64.0%, arabinose 6.0%, galactose 22.0%, glucose 2.6%, and xylose 2.1% (Goycoolea & Cárdenas, 2003). On the other hand, there are differences, types, and composition of the different SDF of the different *Opuntia* species in different parts of these plants and byproducts, as can be observed in Table 13.5.

**Table 13.5** Composition of mucilage and pectin sugars from *Opuntia* spp. plants and fruits

Source	Type of polysaccharide	Composition	References
Cladode, <i>Opuntia dillenii</i>	Mucilage	Rhamnose (15.70%), Arabinose (38.80%), Xylose (5.10%), Galactose (33.00%), Glucose (5.10%), Uronicacida (2.50%)	Kalegowda et al. (2017)
Cladode, <i>O. ficus indica</i>	Mucilage	8% and 12.7% of galacturonic acid and various neutral sugars, such as L-arabinose, D-galactose, L-rhamnose, and D-xylose.	McGarvie and Parolis (1981)
Cladode, <i>Opuntia spinulifera</i>	Mucilage	Arabinose, galactose, rhamnose, and xylose as neutral sugar, D-galacturonic acid	Madera-Santana et al. (2018)
Prickly pear fruit peel, <i>Opuntia albircapa</i> Scheinvar	Pectin	Galacturonic acid, rhamnose, arabinose, galactose and glucose	Lira-Ortiz et al. (2014)
Pear peels, <i>Opuntia ficus indica</i>	Pectin	Rhamnose and galacturonic acid	Majdoub et al. (2001)
Cactus, <i>Opuntia ficus indica</i>	Pectin	Rhamnose, arabinose, galactose, xylose	Cárdenas et al. (2008)

### 3.2 Insoluble Dietary Fiber in *Opuntia* spp.

Insoluble dietary fiber (IDF) usually includes cellulose, insoluble hemicelluloses, lignin, and resistant starch. Regarding total dietary fiber, IDF accounts for a major proportion and exhibits different chemical composition and functional properties (Hua et al., 2019). Cellulose is a polysaccharide consisting of a linear chain of several hundred to over 10,000  $\beta$ ; 1–4 linked D-glucose units and the most abundant organic polymer on earth. On the other hand, hemicelluloses constitute 25–30% of plant materials and the most abundant plant polysaccharides after cellulose. This molecule is complex, branched carbohydrate polymers consisting of different sugars, and within the plant cell walls, they are closely associated with cellulose (Chen et al., 2020).

*Opuntia* spp. naturally contains IDF in different proportions and types. One of the most knowing species of this plant is *Opuntia ficus indica*, which has been widely studied regarding its dietary fiber content, where the results of these studies have demonstrated, for example, that in cactus pear pulp, the IDF is composed of cellulose, hemicellulose, and lignin (El-Kossori et al., 1998). One interesting issue of cladodes or nopal (*Opuntia ficus indica*) is that there is a variation in the IDF content according to age; that is, the IDF is higher in older cladodes, turning these into a better source of insoluble fiber (Hernández-Urbiola et al., 2010). Table 13.6 shows IDF contents in different species, parts, and byproducts of *Opuntia* spp.

**Table 13.6** Content of insoluble dietary fiber (IDF) in different *Opuntia* spp. species

Source	IDF	Reference
Cladode of <i>Opuntia ficus indica</i> (integral cactus tissue)	284.5 g/kg	Reyes-Reyes et al. (2019)
Mucilage of <i>Opuntia streptacantha</i> (cladode, integral cactus tissue)	63.7 g/kg	
Isolated cactus fiber in <i>Opuntia</i> sp. (integral cactus tissue)	512 g/kg	
Pulp of xoconostle fruits, <i>Opuntia joconostle</i> cv. Cuaresmeño	1.47 ± 0.07 g/100 g of fresh weight	Morales et al. (2012)
Seeds of xoconostle fruits, <i>Opuntia joconostle</i> cv. Cuaresmeño	18.85 ± 0.12 g/100 g of fresh weight	
Pulp of of xoconostle fruits, <i>Opuntia matudae</i> , cv. Rosa	1.16 ± 0.01 g/100 g of fresh weight	
Seeds of xoconostle fruits, of <i>Opuntia matudae</i> , cv. Rosa	29.04 ± 0.57 g/100 g of fresh weight	
Prickly pear peel, <i>Opuntia ficus indica</i>	18.36 ± 3.40%	Cruz-Requena et al. (2016)
Korean cactus fruit ( <i>Opuntia humifusa</i> )	13.0 ± 0.08%	Cha et al. (2013)
By-products of cladodes (spines, epidermis) <i>Opuntia ficus indica</i> var. Atlixco	54.45 ± 1.23 g/100 g dry matter	Bensadón et al. (2010)
By-products of cladodes (spines, epidermis) <i>Opuntia ficus indica</i> var. Milpa Alta	53.13 ± 3.72 g/100 g dry matter	
By-products of fruit, green tuna (spines, epidermis and glochids) <i>Opuntia ficus indica</i>	49.95 ± 0.74 g/100 g dry matter	
By-products of fruit, red tuna (spines, epidermis and glochids) <i>Opuntia ficus indica</i>	19.39 ± 1.57 g/100 g dry matter	

## 4 Metabolism of *Opuntia* Fiber in the Digestive System

Dietary fiber is considered an essential component in the human diet (Capuano, 2016). These included substances of plant origin such as carbohydrate polymers of three or more monomeric units and derivatives thereof, which can resist enzymatic hydrolysis in the digestive system reaching intact the colon (CAC, 2008), thus, promoting physiological benefits for the consumer (Sawicki et al., 2017). Those who consume high amounts of fiber in their diets have a lower incidence of developing chronic diseases than the population that manages a diet low in dietary fiber (Capuano, 2016).

The digestive system comprises the mouth, pharynx, esophagus, stomach, small intestine, large intestine, and anus. Through chemical and mechanical processes, the digestive system can digest food until the necessary nutrients are absorbed and transported to the cells (Ascencio-Peralta, 2012). The digestive system's functions are ingestion, secretion, mixing and propulsion, digestion, absorption, and defecation. The digestive system's correct functioning will provide the best use of nutrients and overall health for the body (Mahan et al., 2013).

Digestion begins in the mouth, where food is reduced into small particles through the teeth' action. Saliva makes food to be swallowed and contains enzymes such as salivary amylase and lingual lipase responsible for starting with the digestion of starch and lipids, among others such as lysozyme and lactoferrin (Kulkarni & Mattes, 2014). The next step is swallowing through the esophagus, where through peristaltic movements, the food bolus advances towards the stomach. In the stomach, the decomposition of food is carried out by gastric juice, made up of enzymes and an acidic pH that helps the food disintegrate. Already in the stomach, the food bolus is known as chyme, where it will be emptied into the intestine. It is in the small intestine that the greatest digestion and absorption of nutrients occurs. Due to the action of pancreatic juice (made up of salts, enzymes, and their pH) and enzymes present in the villi of the enterocyte.

Resulting in the hydrolysis of macronutrients to smaller molecules, that is, sugars, amino acids, peptides, and fatty acids (Mahan et al., 2013). All these compounds can be absorbed through the villi that make up the intestine's brush border, thus passing into circulation. Some nutrients cannot pass freely through epithelial cells (passive transport), so it has to be incorporated into different molecules, such as fatty acids, which bind to a micelle and are absorbed through pinocytosis. Digestion is completed in the large intestine, where DF is primarily digested (Capuano, 2016).

The colonic bacterial flora can produce enzymes that can digest compounds such as carbohydrates and proteins, which could not be metabolized in the small intestine (Garcia Peris et al., 2002). This process occurs under anaerobic conditions, which is called fermentation (Escudero & González, 2006). Dietary fibers and carbohydrate polymers undergo bacterial fermentation. Nondigestible carbohydrates are the primary source of energy for intestinal microorganisms (Simpson & Campbell, 2015). The glucose polymers are hydrolyzed to monomers until pyruvate is obtained through the Embden-Meyerhoff metabolic pathway. Pyruvate is converted to short-chain fatty acids (AGCC), mainly acetate, propionate, and butyrate. In addition to the production of gases such as hydrogen, carbon dioxide, nitrogen, and methane (Escudero & González, 2006).

Although it is known that the fiber reaches the colon intact, it undergoes structural and chemical changes throughout the digestive tract. In the oral cavity, the teeth' mechanical action causes the cell wall to fragment, making the contact surface smaller to improve the bioavailability of nutrients (Hardacre et al., 2014). The fiber absorbs water in the stomach and intestine, swelling in a greater or lesser proportion depending on its hydration capacity. Going through partial solubilization, depending on the type of fiber. Solubilization is limited when the fiber is still part of the cell wall structure due to chemical interactions with other components (Ulmus et al., 2012).

Similarly, it is mentioned in the literature that DF retains its chemical structure during digestion; however, limited hydrolysis can occur if the gastric pH remains low. It resulted in energy production of between 1 and 2.5 cal/g (Garcia Peris et al., 2002). It is worth mentioning that colonic bacteria degrade more than 50% of the fiber consumed; the rest is eliminated through the feces (Escudero & González, 2006).

Depending on the type of fiber, it is the ratio in which bacteria can ferment them. In addition to the fiber's different physicochemical characteristics, such as solubility and viscosity, this ratio influences its digestion. It is the soluble fiber, the one with the highest fermentability capacity. Fibers highly fermentable with high solubility and viscosity participate in glucose metabolism, causing it to be absorbed slowly (McCorie & Fahey, 2013). In the case of *Opuntia* spp., the pectins and mucilages present (soluble fiber) makes its high solubility and viscosity, produce effects on glycemic control and reduction of blood cholesterol (Gouws et al., 2020). Intestinal microorganisms cannot fully ferment insoluble fiber, but they increase intestinal transit rate, thus reducing exposure to bacterial fermentation in the colon. It also participates in water retention, increasing fecal volume, and the daily number of bowel movements (Liu et al., 2016; Mahan et al., 2013). The degree of polymerization and solubility of complex carbohydrates modifies the location of fermentation in the gastrointestinal tract. Soluble fibers are metabolized in the proximal part of the intestine (ileum and ascending colon). However, the less soluble fibers are partially fermented in the distal colon, where intestinal transit is slower and bacterial concentrations are higher (Holscher, 2017).

Substrate availability and microbiota diversity are also influenced by pH, host secretions, and transit time. In this way, an increase in the byproducts' concentration resulting from fermentation is seen, such as the AGCC (Sawicki et al., 2017). The AGCC is absorbed in 90% by the colonocyte, using butyrate as the main energy source, metabolizing it to carbon dioxide, ketone bodies, and water. It is worth mentioning that not all fibers produce the same amount of AGCC. All substrates produce acetate as the final product of fermentation with variations in propionate and butyrate (De Vadder et al., 2014). Propionate is used as a substrate for the gluconeogenesis pathway, and acetate will be metabolized, causing glutamine and ketone bodies, being used as the main energy substrate in the enterocyte. An excessive amount of fermentable fiber in the colon can cause increased gas production, bloating, pain, flatulence, decreased colon pH, or even diarrhea (Holscher, 2017).

## 5 Beneficial Health Effect of Insoluble Dietary Fiber

For years, fiber has been considered an essential part of human nutrition, finding multiple benefits for weight control and heart health maintenance, as it works preventively against diseases (Zielinski & Rozema, 2013). The edible part of plants known as DF has been found and has been associated with foods such as cereals, fruits, or fibrous vegetables. However, research has gone deeper into this subject, as fiber has been implicated in several beneficial physiological effects. The reduction of cholesterol and glucose in the blood stands out (Daou & Zhang, 2013). It should be clarified that not all types of fiber are equal. It is classified according to its solubility with water as insoluble fiber (IF) or soluble fiber (SF). So, the health benefits of consuming insoluble dietary fiber are discussed below. The highest content of the insoluble fraction of DF is found mostly in cereals; this is compared to other sources

such as vegetables (Tejada-Ortigoza et al., 2016). The association between disease risk decrease is more closely linked to cereal fiber (Satija & Hu, 2012). Table 13.7 shows the description of the main components of insoluble fiber and its source.

The epidemiological studies showed that people with diets high in fat, sugar, salt, and low consumption of foods like vegetables, are more likely to suffer from chronic diseases such as diabetes, obesity, heart disease, and cancer. Based on these antecedents, there is a need to know the components and benefits, including daily diet and knowing each group of people (Williams et al., 2019).

**Table 13.7** Main components of insoluble fiber (IF), health benefits and source of production

IF	Description	Sources	Reference
Cellulose	Main component found in the cell wall of plants. It is located within a matrix of hemicellulose, pectin and lignin. Most abundant natural biopolymers available. Resistant to digestive enzymes May have up to 10,000 units of packaged, unbranched glucose. Can be fermented in the large intestine by microbiota, which produces short-chain fatty acids (SCFA).	It's built around: One quarter of the dietary fiber in grains and fruits, and one third in vegetables and nuts. The main source of cellulose is wheat bran. It is also present in legumes and nuts.	Fuller et al. (2016) Ciudad-Mulero et al. (2019)
Hemicellulose Non-starch polysaccharides	Non-cellulose compound. They're smaller. Cell wall polysaccharides, with spines of glucose units with $\beta$ -1,4 glycosidic bonds. They have a variety of sugars and are branched. They are usually grouped into four classes: xylans, xyloglucans, glucomannans and the mixed linkage of $\beta$ -glucans.	It contains xylose and some galactose, mannose, arabinose among other sugars. In the cell walls of cereal grains including oats and barley. Also present in fruits, legumes and nuts.	Fuller et al. (2016) Wong et al. (2016) Ciudad-Mulero et al. (2019)
Lignin	Random complex polymer. It is chemically linked to the hemicellulose in plant cell walls. It is a polymer of phenylpropane units. It is an aromatic, hydrophobic polymer. Lignin molecules vary in molecular weight and methoxyl content.	Foods with a woody component, e.g. the outside of cereal grains. It is found in the outer layers of some bran.	Fuller et al. (2016) Wong et al. (2016) Ciudad-Mulero et al. (2019)



The components of DF, mainly from vegetables, fruits, and whole grains, have been mentioned to reduce the risk of different cancer types such as colon, breast, and prostate (Devi et al., 2011). Phenolic compounds have been directly linked to having antioxidant, anti-mutagenic, anti-estrogenic, anticancer, and anti-inflammatory effects which decrease and/or prevent certain diseases (Devi et al., 2011). Insoluble dietary fiber (IDF) comes from these compounds (Ciudad-Mulero et al., 2019). Besides, IDF has been linked to the intestinal tract's proper functioning by increasing the weight and/or size of the stool and reducing intestinal transit time, contributing to preventing constipation and diverticulosis (Ciudad-Mulero et al., 2019).

There is little information about insoluble fiber's effects, including cellulose, on human health (Fuller et al., 2016), while nutritional lignin is mostly inactive. Studies on humans' physiological effects are very scarce (Fuller et al., 2016). Among the benefits of fiber on the human body, most of them refer to the gastrointestinal system, mainly preventing colon cancer. Cellulose decreases the excretion and/or production of certain mutagens in the feces and concentration of bile acids in the human body (Fuller et al., 2016). Lignin, in turn, increases stool volume and reduces fecal concentrations of secondary bile acids and 4-cholesten-e-one (potential cancer-causing agents), stimulating intestinal transit (Ciudad-Mulero et al., 2019), having a greater capacity to retain water, and providing technological benefits (Daou & Zhang, 2013). Besides, hemicellulose increases the hydration of the stool so that bowel movements occur more regularly. It is preventing cholesterol from being absorbed. This fiber is digested by the microbiota, increasing the number of beneficial bacteria and producing CFA, an energy substrate for colon cells (Ciudad-Mulero et al., 2019; Daou & Zhang, 2013). These health benefits of fiber are important to consider. Fiber needs to gel to stay in good condition. Poorly fermented coarse insoluble fiber (wheat bran) irritates the intestine's mucous membrane, causing water to be stimulated and mucous to be secreted, which in turn would cause an increase and soften the stool (McRorie, 2017). Dietary fiber prevents diseases such as type 2 diabetes, cardiovascular disease, gastrointestinal cancers, among other disorders. Since DF traps dietary compounds that are toxic and, therefore, harmful to the human body, producing short-chain fatty acids in the intestine (serve as substrates for colonocytes) and regulating specific proteins that are involved with cancer progression (Devi et al., 2011).

*Opuntia* fiber has been tested on several cell lines such as HT-29 and Caco2, which are very resistant and susceptible to apoptosis (Del Socorro et al., 2017). It has soluble and insoluble fibers and polyphenols, which modulate the expression of inflammatory genes and oxidative stress, which modify the intestinal microbiota (Sanchez-Tapia et al., 2017). Oxidative stress is the main factor in the physiopathology of type 2 diabetes mellitus and heart disease as it accelerates the development of arterial lesions forming atherosclerosis. *Opuntia* tissue is an inhibitor in the oxidative environment, due to its antioxidant components, antihyperglycemic, and anti-diabetic properties, preventing the development of diabetes (Del Socorro et al., 2017). *Opuntia* tissue has a high biotechnological potential and has been used for centuries for the treatment of diseases such as obesity, diabetes, gastric, cardiac, and

inflammatory ulcers, because of the presence of antioxidants, pigments, or phenolic acids (Del Socorro et al., 2017).

## 6 Beneficial Health Effect of Soluble Dietary Fiber

The dietary fibers are heterogeneous. They have different physicochemical properties like solubility, viscosity, and fermentability that influence their therapeutic effects (Thompson et al., 2017). Soluble and insoluble fibers make up the two basic categories of DF. The soluble fiber is localized in the cell and is not part of structural polysaccharides like pectin, gum, and mucilage (Del Socorro et al., 2017). The soluble fiber increases the viscosity of the stomach content; this capacity has been associated with prolonged gastric emptying, a reduction of the intestinal transit, rate of starch digestion, and glucose absorption. These effects cause modifications in blood glucose and cholesterol concentrations (Grundy et al., 2016). Other properties attributable to soluble fiber intake are increased satiety, improved blood lipids concentrations, and glycemic response (Thompson et al., 2017). *Opuntia* spp. tissue has a high nutritional value, mainly due to its mineral, protein, DF, and phytochemical contents (Ventura-Aguilar et al., 2017). Several studies have shown a beneficial effect on the health of the soluble fiber from *Opuntia* spp.

### 6.1 Effects in Weight Control

Obesity is a heterogeneous disorder characterized by excessive fat accumulation in the body. It is a complex disorder that involves genetics and environmental factors. Estimations by the World Health Organization (WHO) indicate that 1.9 billion are overweight and obese worldwide. Obesity is ligated with several comorbidities like type 2 diabetes mellitus, atherosclerotic cardiovascular disease (ASCVD), and chronic kidney disease (Dagpo et al., 2020).

The soluble DF from *Opuntia* spp. have been used in experimental and clinical assays (Table 13.8). In a clinical trial in healthy subjects for 45 days, the volunteers that received a cactus fiber tablet showed an increased fecal fat excretion compared with placebo, without adverse effects. This finding could be due to DF components such as mucilage, gum, and pectin (Uebelhack et al., 2014). Otherwise, the Litramine IQP G-002AS compound, a fiber complex from *Opuntia ficus-indica*, can bind to dietary fat, forming a non-absorbable complex fat-fiber is eliminated by feces. In a clinical trial with 12 weeks, subjects with obese and overweight were treated with IQP G-002AS. The investigation results showed that most patients lost at least 5% of their initial body weight and reduced BMI, body fat composition, and waist circumference (Grube et al., 2013).

Sanchez-Tapia and coworkers demonstrated attenuation in body weight gain in rats fed with high fat-high sucrose (HFS) diet by the effect of nopal consumption.

**Table 13.8** Experimental and clinical trials with the soluble dietary fiber of *Opuntia* spp.

Genus	Plant material	Type of study	Beneficial effect	Modification of measures evaluated	Reference
<i>Opuntia ficus indica</i>	Dehydrated nopal cladodes	Experimental. Obese rats fed high fat/sucrose diet	Protects from metabolic endotoxemia	Modifying gut Microbiota, restoration of the mucus layer and increase occludin	Sanchez-Tapia et al. (2017)
<i>Opuntia ficus indica</i>	Whole fresh liquefied nopal cladodes and mucilage	Experimental. Male Wistar rats on a high-fructose diet	Triglycerides and arterial pressure-lowering action	Lower levels of triglycerides and diastolic arterial pressure	Cárdenas et al. (2019)
<i>Opuntia ficus indica</i>	Cactus fiber tablet	Clinical investigation. A double-blind, randomized, placebo-controlled, cross over study in healthy subjects	Body weight reduction	Cactus fiber promotes fecal fat excretion in healthy adults	Uebelhack et al. (2014)
<i>Opuntia ficus indica</i>	Natural fiber complex enriched with additional soluble fiber from <i>Acacia</i> spp. (Litramine IQP G-002AS)	Clinical investigation. A double-blind, randomized, placebo-controlled study	Body weight reduction	Reduction in BMI, body fat composition, and waist circumference	
<i>Opuntia robusta</i>	Broiled edible pulp of prickly pear	Clinical investigation. Patients from familial hypercholesterolemia	Cholesterol-lowering effect	Reduction in total and LDL-cholesterol	Budinsky et al. (2001)
<i>Opuntia streptacantha</i>	Broiled nopal stems	Clinical investigation. Patients with type II diabetes, treated with diet alone, or in combination with sulfonylureas	Hypoglycemic effect	Reduction in glucose levels	Frati-Munari et al. (1988)

(continued)

**Table 13.8** (continued)

Genus	Plant material	Type of study	Beneficial effect	Modification of measures evaluated	Reference
<i>Opuntia ficus indica</i>	Capsules of cladode and fruit skin extract (OpunDia™)	Clinical investigation. A double-blind randomized cross-over study	Insulin stimulation	Increase serum insulin concentration while reducing blood glucose level	Deldicque et al. (2013)
<i>Opuntia ficus indica</i>	Flour of cladodes	Experimental study. Streptozotocin-induced diabetic rats	Hypoglycemic effect	Reduction of postprandial blood glucose	Nunez-Lopez et al. (2013)
<i>Opuntia ficus indica</i>	Dehydrated nopal cladodes	Experimental study. A model of male obese Zucker rats	Hepatic steatosis	Reducing steatosis and oxidative stress	Moran-Ramos et al. (2012)

<sup>a</sup>BMI body mass index

The authors also noted a change in the gut microbiota. These effects could be due to the soluble and insoluble fiber content. Besides, the HFS diet reduced the intestinal mucus layer compared with the control group; nopal consumption restored the mucus layer and increased the expression of occludin-1, important in the maintenance of the tight junctions in epithelial cells of the intestine. The authors suggested using the nopal as a functional food and prebiotic for persons with obesity and related abnormalities (Sanchez-Tapia et al., 2017).

These beneficial effects could be due to *Opuntia* cladodes, a source of DF, which may reduce body weight because they are considered hypolipidemic. The soluble DF (low methoxyl pectin) and calcium content of cladodes are considered important elements for their physical properties (Del Socorro et al., 2017). The mucilage is also important, constitutes 14% of the cladodes' dry weight, and has a high water-holding capacity, which contributes to the high fiber content of the cactus stem (Ventura-Aguilar et al., 2017).

## 6.2 Effects on Glucose Reduction

Diabetes is a highly prevalent disease and is one of the major public health concerns. In addition to the genetic and environmental factors, risk components ligate to diabetes include age, race/ethnicity, obesity, physical inactivity, high blood pressure, and sedentary lifestyle (Gudi, 2020). Frati-Munari et al. (1988) described the hypoglycemic effects of *Opuntia streptacantha* Lem. in patients with type 2 diabetes. After the ingestion of 500 g of broiled nopal stem, serum glucose and serum insulin levels decreased significantly. Another study showed the insulinogenic

action of *Opuntia ficus indica* cladode in healthy young volunteers' post-exercise. As a result, *Opuntia* reduced blood glucose and increased serum insulin concentration compared to the placebo (Deldicque et al., 2013). At the same time, Nunez-Lopez et al. (2013) studied the relation between the maturity stage of cladodes from *Opuntia* and its anti-diabetic properties. They used cladode flours for testing the hypoglycemic effect in a streptozotocin-induced diabetic rat model. The flours of small and medium cladodes (20 and 30 days, respectively) showed the most significant impact on reducing glucose. In this study, the viscosity is a factor that influences the anti-diabetic effects. Also, the hypoglycemic of the nopal may be due to its fiber content, mainly pectin.

### 6.3 Effects Related to the Cardiovascular System

Worldwide, cardiovascular diseases (CVD) are the leading cause of death and encompass various diseases like vasculature, myocardium, cardiac electrical circuits, and cardiac development (Liu et al., 2020). Circulating lipoproteins molecular markers in CVD risk management due to their frequent accumulation and associated risk of atherosclerotic plaques (Gouws et al., 2020). Budinsky and coworkers showed that the DF in prickly pears from *O. robusta* lower the cholesterol levels in hyperlipidemic nondiabetic human patients. The protective effect of prickly pear may result from pectin, a soluble fiber. The authors consider that *Opuntia robusta*'s regular ingestion is an interesting nutritional option as an auxiliary in treating patients with hypercholesterolemia (Budinsky et al., 2001). Another study evaluated mucilage fiber's effect in an *in vivo* model in rats with a high-fructose diet for 30 days. A reduction in triglycerides and diastolic arterial pressure than control has been demonstrated by the effect of nopal and mucilage administration. The authors suggest that the mucilage could be responsible for these effects, but the mechanisms are still unknown (Cárdenas et al., 2019).

### 6.4 Hepatoprotective Effects

Hepatic diseases are one of the main threats to public health worldwide. The liver has a fundamental role in metabolism, secretion, storage, and detoxification of endogenous and exogenous substances. In the search for alternatives for liver disease treatment, plants' use is an interesting option (Madrigal-Santillan et al., 2014). Moran-Ramos et al. (2012) showed the effects of *Opuntia ficus* on hepatic steatosis. In the obese Zucker murine model, which mimics the hepatic pathology of nonalcoholic fatty liver disease (NAFLD), the consumption of nopal showed a remarkable attenuation (~50%) of hepatic triglyceride, reduction in hepatomegaly, and biomarkers of hepatocyte injury (ALT, AST). The hepatic attenuation was observed because of the higher serum concentration of adiponectin and by increasing VLDL

secretion. Besides, the hepatic reactive oxygen species and lipoperoxidation biomarkers were lower by the effect of nopal consumption compared to control. The authors suggest that other bioactive components in the nopal, such as dietary fiber, may also be involved. Previous works have been described that soluble fibers modify hepatic lipid synthesis and improve intestinal permeability, thus improving NAFLD biomarkers.

## 7 Potential Agro-Industrial Use of *Opuntia* Dietary Fiber

Dietary fibers have technological properties that can be used during food formulation, such as in the modification of texture, consistency, rheological behavior, and providing antioxidant properties and improving the stability of foods during their production storage (Dhingra et al., 2012). The current tendency is to find new sources of DF and natural antioxidants, which come from agricultural byproducts traditionally undervalued. *Opuntia* plants can be an essential source of DF and antioxidants within the same matrix and can have multiple industrial applications (Ayadi et al., 2009). The *Opuntia* fruits and cladodes contain many polysaccharides such as pectins, mucilages, hemicelluloses, and cellulose. These polysaccharides are the main components of dietary fiber and have a wide range of nutraceuticals and nutritional applications (Arias-Rico et al., 2020; Ciriminna et al., 2019).

Dietary fibers from different sources have been used to replace wheat flour in the preparation of bakery products. Ayadi et al. (2009) reported the use of cladode powder from *Opuntia ficus* as a source of DF in bread making. The cladode powder's addition caused a change in the dough's texture by decreasing its elasticity and causing a slight green color in the bread; therefore, only pieces of bread containing a 5% level of cladodes flour presented favorable results in the preparation of bread. In another study carried out by Bouazizi et al. (2020), flour from *Opuntia ficus* fruit shells was incorporated as an innovative ingredient in biscuits formulation. The biscuits prepared with 20–30 g/100 g *Opuntia* flour had a higher content of DF and more appreciated for their smell, color, and flavor than those that did not contain *Opuntia* flour, aside from the richness of active and functional biomolecules. In addition to the use of flour from *Opuntia*, mucilage has also been used in substitution to water in bread production. Liguori et al. (2020) reported that *Opuntia ficus* mucilage enriched bread with DF and other bioactive components such as antioxidants, without affecting the fermentation process and maintaining the properties of the dough and bread. Therefore, *Opuntia* components, such as cladode, fruit peel, or mucilage, can be a viable alternative for the production of bakery products enriched with DF and benefit human health.

The use of the whole fruit of *Opuntia xocconostle* (shell, fruit, and seed) has also been reported as an essential source of DF and antioxidants. It could have great applications in the pharmaceutical and food industries. One of the main uses is the capacity of glucose retention by the DF of xocconostle, which is related to a hypoglycemic effect. Therefore, its application in preparing functional foods for patients

with type 2 diabetes mellitus is proposed (Pimienta-Barrios et al., 2008). Likewise, the formulation of tablets added with powder from residues (mesocarp and pericarp) of green and red fruit of *Opuntia ficus-indica* has been described that can be an interesting product for the food supplement industry, this due to the high content of DF (mainly insoluble fiber and high molecular weight mucilages) and antioxidants that *Opuntia* residues possess (Manzur-Valdespino et al., 2020).

Another application is the use of *Opuntia* mucilage to develop protective covers for fruits and vegetables to extend its shelf life. It has been reported that the use of mucilage as a protective layer extends the shelf life of strawberry and guava, without affecting its sensory properties such as odor, color, and flavor. Besides, strawberries showed better firmness; this provides better resistance to mechanical damage during storage (Del Valle et al., 2005; Zegbe et al., 2015). Therefore, the use of mucilage as an ingredient in food protection layers can be an effective alternative due to its low cost, availability, and effectiveness (Gheribi & Khwaldia, 2019).

## 8 Future Trends

The *Opuntia* genus is important because of its excellent nutritional value and functional properties (Figuroa-Cares et al., 2010). In addition to its insoluble and soluble dietary fiber (Nair et al., 2010), its composition of sugars (L-arabinose, L-rhamnose, D-xylose, and D-galactose), phenolics (Cortez-García et al., 2015), vitamins (C, and E), and betalains (yellow betaxanthines and red betacyanins) (Albano et al., 2015), make this plant focus of different investigations about its benefits on human health and applications to promote better use of this plant.

There are currently different industrial uses of prickly pear and nopalitos because these products are cheap and profitable. Therefore, *Opuntia* tissue has been used in various biotechnological procedures to obtain pigments, sweeteners, wines, liqueurs and distillates, fermented juices, nectars, edible oil, flours, cheeses, marshmallows, gelling agents, and thickening additives (Monrroy et al., 2017), among other minimally processed products (Valdez et al., 2008). Furthermore, *Opuntia* tissue has applications in the cosmetics industry to produce masks, gels, conditioners, shampoo, lotions, and creams (Corrales-García & Flores-Valdez, 2000). However, although *Opuntia* has currently widely used in the industry, the areas of interest that have gained importance are the epidemiological and pharmacological areas since *Opuntia* is a good source of DF that has potential in human health. It is also considered an important natural source of bioactive compounds with importance in medicine (Sáenz et al., 2004). Since several studies mention that a higher intake of DF such as that of *Opuntia* is associated with the risk reduction of different diseases including diabetes type 2, cancer, obesity, and cardiovascular diseases (CVD), because of fiber potential mechanisms such as satiety effect, induced production (intestinal microflora) of short-chain fatty acids with anti-inflammatory and immunomodulatory properties, fiber fermentation by microorganisms such as *Butyrivibrio fibrisolvens* promotes limited absorption of LDL cholesterol and glucose, retention

of bile acids and ingestion of bioactive compounds (Veronese et al., 2018). Various experimental and clinical trials support the fiber effects on health. Han et al. (2017) consigned that when *Opuntia humifusa* extracts (CE) at concentrations of 3% (L-CE group) and 6% (H-CE group) were administrated for 25 days to mice with Loperamide-induced constipation, reduced constipation and improved fecal granules, water content, and increased distal colon were observed by increasing mucus content in epithelial cells. Other study indicated that the positive benefits of DF are by altering the function and composition of the intestinal microbiota, helping to improve the immune system, thereby reducing the risk to some types of cancer, such as pancreatic, colorectal, renal, gastric, esophageal, renal, endometrial and breast cancer (Kuo, 2013). For its part, the fiber viscosity improves glycemic control and cholesterol concentration (Ho et al., 2017). Finally, consumption of DF increases the intake and bioavailability of minerals, vitamins, and bioactive compounds (Veronese et al., 2018); as mentioned by Bensadón et al. (2010). Dietary fiber and bioactive compounds are of great interest for food processing since they are used as functional ingredients. However, despite obtaining various products derived from the prickly pear and nopalis, there are still no plantations that are fully dedicated to satisfying the demanded volumes for industrialization, so this would be an excellent opportunity for *Opuntia* producers (Jorge & Troncoso, 2016).

The phenolics (flavonoids and phenolic acids) content of prickly pear and nopalis have high antioxidant potential, which helps to eliminate free radicals, prevent degenerative diseases, and have anti-inflammatory and anticancer effects, and assisting in cardiovascular dysregulation (El-Mostafa et al., 2014), strokes, cataracts and Alzheimer's (Jorge & Troncoso, 2016). Because of its nutritional value and bioactive compounds content that favors a healthy state; more *Opuntia* fiber ingestion is recommended by the diet (Veronese et al., 2018). However, more studies on *Opuntia* DF are needed to confirm the findings on preventing diseases such as cancer, degenerative and cardiovascular diseases.

As a result of *Opuntia* industrialization are generating massive amounts of waste, which become a serious environmental problem, it is difficult and expensive to get rid of *Opuntia* fruits shells (Abarca et al., 2010), even though these residues are promising sources of DF (Sears et al., 2013) which can be used as new and cheaper sources of fiber, in comparison to that fiber obtained from other sources such as cereals (Abarca et al., 2010), as well as a source of mucilage (soluble fiber) that has the property of forming viscous or gelatinous colloids (Saenz, 2006). Although the problem in the use of these residues could lie in the lack of adequate techniques to extract the compounds present in the shells, seeds or the use of mucilage, this could lead to the search for innumerable new procedures and chemical substances that could be used in different industrial or pharmacological processes (Sears et al., 2013). An example of this would be using the *Opuntia ficus-indica* mucilage as a natural flocculant for water clarification, removing heavy metals and agglomerating pathogenic microorganisms. *Opuntia* mucilage is a cheap and clean flocculant and may replace the inorganic flocculants (sodium aluminate, ferric chloride, aluminum sulfate, ferric, and ferrous), which are more expensive (Contreras Lozano et al., 2015). On the other hand, the aqueous extract of *Opuntia* mucilage could be used as



a reducing and stabilizing agent during biosynthesis of zinc oxide nanoparticles (NPs-ZnO) or other nanomaterials (Francisco-Escudero et al., 2017), with potential of pharmacological or antimicrobial uses (El-Mostafa et al., 2014). This type of biosynthesis of nanomaterial with biological activities has gained significant interest (El-Seedi et al., 2019; Yoosefian et al., 2019).

On the other hand, El-Mostafa et al. (2014) indicated that in a study using mice, *Opuntia* mucilage stabilized the damaged gastric mucosa's plasma membranes. In humans, mucilage decreased hangover symptoms. The use of agricultural residues helps to reduce the adverse effects on the environment. At the same time, these residues could generate agroecological alternatives and new agro-industrial products to generate new sources of income for producers (Cabrera-Garcia et al., 2018).

## 9 Conclusions

*Opuntia* has many industrial applications, especially in the food and pharmacological areas, because it is an excellent DF source, represented by mucilage and pectin. Mucilage is composed of carbohydrates with highly branched structures and galacturonic acid in different proportions. Insoluble dietary fiber includes cellulose, insoluble hemicelluloses, lignin, and resistant starch, while soluble fiber is integrated by pectin and mucilage. *Opuntia* dietary fiber produces effects on glycemic control and the reduction of blood cholesterol. Intestinal microorganisms cannot fully ferment insoluble fiber. However, it increases intestinal transit rate and participates in water retention, increasing fecal volume, and the daily bowel movements. Dietary fiber prevents diseases such as type 2 diabetes, cardiovascular disease, gastrointestinal cancers, among other disorders, and has technological properties that can be used during food formulation. However, it is necessary to continue studying different *Opuntia* species and their effects on dietary fiber to increase its added-value as a functional food.

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# Chapter 14

## *Opuntia* spp. Chemical Constituents and Bioactive Compounds, with Particular Regards to Polyphenols



Alessandra Durazzo, Massimo Lucarini, Amirhossein Nazhand, Antonio Raffo, Eliana B. Souto, Ginevra Lombardi-Boccia, Antonello Santini, and Elisabetta Lupotto

**Abstract** The plant-based biologically active ingredients are commonly molecules with therapeutic activities for various medical conditions. *Opuntia* spp., belonging to the family *Cactaceae*, contain a high level of bioactive compounds, which deserved attention in the past decade to evaluate the bioactivity of a whole extract or a specific purified compounds. This chapter aims to describe the chemical constituents and bioactive compounds in *Opuntia* spp., notably polyphenols, phenolic acids, flavonoids, organic acids, and their beneficial properties.

**Keywords** Bioactive compounds · Phenolics · Phenolic acids · Flavonoids · Organic acids

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## 1 Introduction

A “bioactive compound” is defined as a compound occurring in nature that can interact with one or more compounds of the living tissue by showing an effect on human health (Biesalski et al., 2009). The first step for determining the beneficial effects of foods on human health is the concerted and combined action of compounds of nutritional and nutraceutical interest. Beyond essential nutrition, the dietary components possess beneficial roles that lead to the advancement of food perception as functional and nutraceutical. The monitoring of the distribution of compound classes in food groups and at different food chain stages represents a significant challenge (Durazzo & Lucarini, 2019).

Plants with high levels of bioactive compounds are good candidates for treating many diseases in humans, which indicates the need to understand the activities and benefits of nutrients and bioactive compounds of medicinal plants. *Opuntia* spp. are rich in different bioactive compounds with diverse therapeutic effects for the treatment of a variety of diseases reported in many studies and review articles (Cota-Sánchez, 2016; Slimen et al., 2016; Del Socorro Santos Díaz et al., 2017; Aruwa et al., 2018; González-Stuar & Rivera, 2019; Barba et al., 2020). Feugang et al. (2006) published a review article regarding the bioactive compounds extracted from the fruit and cladode extracts of *Opuntia* spp. and reported the presence of betalains, amino acids, minerals, mucilage, and pectin. Another review article reported the presence of amino acids, polyunsaturated fatty acids (PUFA), and polyphenols in different parts of *Opuntia* spp. with various therapeutic activities such as metabolic syndrome, rheumatism, cancers, and virological and bacterial infections (El-Mostafa et al., 2014).

Aragona et al.’s (2018) reviewed on *Opuntia ficus-indica* (L.) Miller, one of *Opuntia*’s main known species, as a source of bioactive compounds for health and nutrition. Ramírez-Ramos et al. (2018) evaluated the prickly pear fruit’s chemical compositions, accounting betalain at the highest level (39.9 mg 100 g<sup>-1</sup> FW) and reporting antioxidant activity. Serra et al. (2013) showed antiproliferative activity of fruit juice from *Opuntia* spp. by arresting cell cycle at various checkpoints (G1, G2/M, and S), in addition to the inhibition of cancer cell growth. Milán-Noris et al. (2016) reported how prickly pear peel extract increased cholesterol excretion and reduced hepatic cholesterol in male hamsters. González-Ponce et al. (2016) reported that feeding Wistar rats with cactus fruit extract (800 mg/kg/day) reduced cell necrosis and LDH leakage.

In the study of Zourgui et al. (2020), the maximum radical scavenging activity (IC<sub>50</sub> = 0.22 mg/mL) was reported for the phytochemicals extracted from peel of *Opuntia* spp. fruit, besides, the antimicrobial effects against *Micrococcus luteus*, *Fusarium oxysporum* and *Staphylococcus aureus*. Aboura et al. (2017) prescribed *Opuntia ficus-indica* (cladodes) plus *Ceratonia siliqua* (carob) in mice for a month. They found an anti-inflammatory activity by declining expression of pro-inflammatory cytokines of IL1b, IL-6, and TNF- $\alpha$  in the spleen, colon, and adipose tissues. Cytotoxic effects of ethanolic and aqueous extracts of *O. dillenii* cladode

controlled the colon cancer cell lines (Caco-2) with  $IC_{50}$  value of 40 and 50  $\mu\text{g/mL}$ , respectively (Lataief et al., 2021).

## 2 Chemical Composition and Bioactive Constituents in the Plant and Waste of *Opuntia* spp.

Prickly pear (*Opuntia ficus indica* (L.) Mill.) is a rich source of vitamins C, B1, B2, A, and E, minerals such as calcium, potassium, magnesium, iron, and phosphorus, as well as bioactive substances, i.e., carotenoids, betalains, and phenolic compounds. Of these, the phenolic acids, betalains, and flavonoids are notable in that they are mostly responsible for this plant's health-promoting properties. Table 14.1 reported updated studies on main bioactive compounds in different parts of *Opuntia* spp. and in Fig. 14.1, the chemical structures of the main compounds are showed. In Fig. 14.2, a focus on betalains structures was reported.

The different plant parts (flower, seed, cladodes, fruits) of *Opuntia* are rich in phenolics such as phenolic acids, flavonoids, organic acids. Cha et al. (2013) reported a high level of antioxidant activity for bioactive compounds of Korean cactus (*Opuntia humifusa*, OH) fruit extract, including phenolic acids (ferulic and protocatechuic acids), and flavonoid (myricetin and taxifolin). A study employed liquid chromatography coupled to mass spectrometry (LC-MSn) method and

**Table 14.1** Main bioactive compounds in different part of *Opuntia* spp.

Part	Bioactive compound(s)	Properties	Reference
Fruit	Phenolics, betalains and ascorbic acid	anti-inflammatory and antioxidant	Gómez-Maqueo et al. (2019)
Fruit	Betacyanins	Hepatoprotective effect	González-Ponce et al. (2016)
Seed	Catechin, epicatechin, and ferulic acid,	Antioxidant	Khaled et al. (2020)
Cladodes	Isorhamnetin tri- and diglycoside, 3-O-methyl quercetin, 3-O-methyl kaempferol and luteolin	Antioxidant	Antunes-Ricardo et al. (2017)
Cladodes	Piscidic acid	Antioxidant	Petruk et al. 2017
Cladodes and fruits	Chlorogenic acid, ferulic acid, gallic acid, quercetin, kaempferol	Antidiabetic, antimicrobial and cytotoxic activity	Abd El-Moaty et al. (2020)
Flower	Isorhamnetin glycosides	Antioxidant, antibacterial	Ammar et al. (2015)
Peel	Betalain compounds	Antidiabetic, antimicrobial and cytotoxic activity	Melgar et al. (2017)

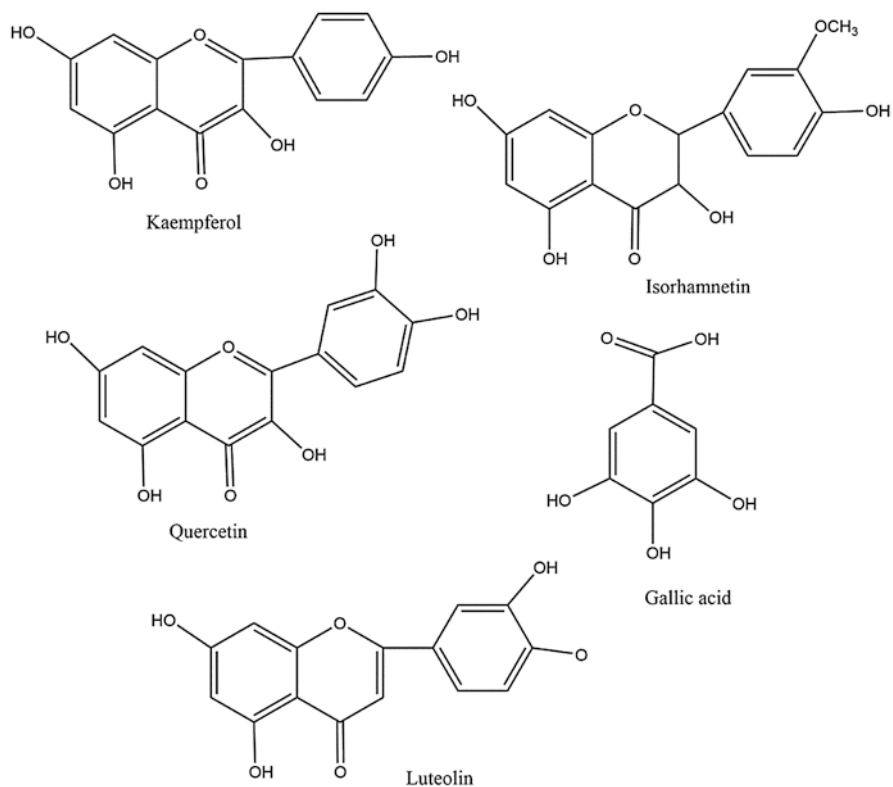


Fig. 14.1 Structure of main bioactive compounds in *Opuntia* spp.

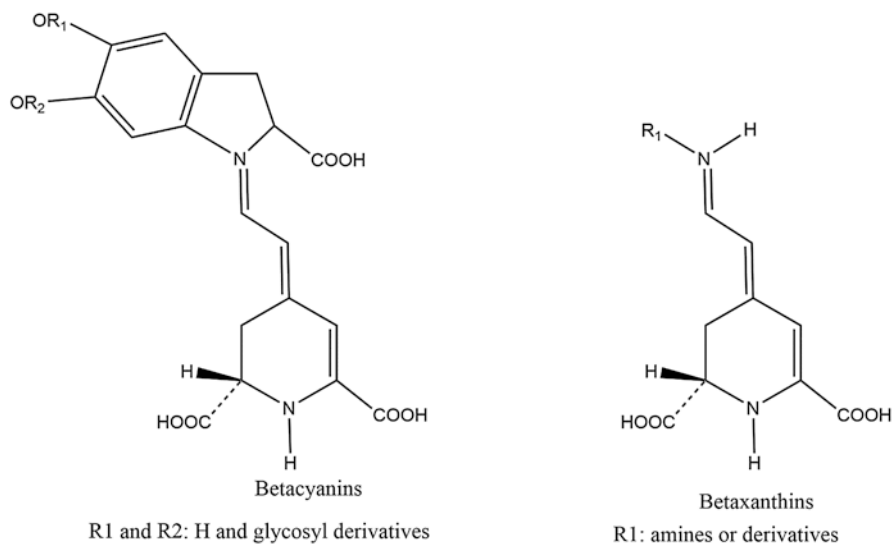


Fig. 14.2 The structure of betalains family

detected phenolic compounds of feruloyl-sucrose isomer 1,2,3 and sinapoyl-diglucoside present in *Opuntia ficus-indica* seeds, and found antioxidant activity using nuclear magnetic resonance (LC-NMR) analysis (Chougui et al., 2013). Abou-Elella and Ali (2014) used MS, IR, <sup>1</sup>H-NMR, and <sup>13</sup>C-NMR techniques to identify total phenolic compounds (17-hydroxy betanin and betanin) of *Opuntia ficus-indica* fruit peel extract and subsequently reported the anticancer potential with an efficiency of 51.5–76.0 dead cells/100 µg/mL.

García-Cayuela et al. (2019) applied HPLC-DAD-ESI-QTOF technique to determine phenolic compounds' chemical compositions, including flavonoid glycosides (kaempferol, quercetin, and isorhamnetin) and piscidic acid extracted from *Opuntia ficus-indica* L. Mill. According to their results, the maximum level of phenolic compounds (49 µg/g) was found in the Spanish Morada cultivar's dry peel.

The prickly pear is a rich source of betalains pigment that is grouped into two classes based on light-absorption and structural features, betacyanin (in reddish-violet colour) and betaxanthin (in yellow-orange color) (Gandía-Herrero & García-Carmona, 2013). Betalamic acid is the structural unit of all betalains, as the starting point to form betalains *via* a Schiff condensation with free amine groups (betaxanthins) or indoline-containing structures (betacyanins) (Naseer et al., 2019). There are reported different bioactivities for the betalains, including anticancer, antidiabetic, antioxidant, and anti-inflammatory properties (Carillo-López & Yahia, 2017; Rahimi et al., 2019; Smeriglio et al., 2019; Palmeri et al., 2020).

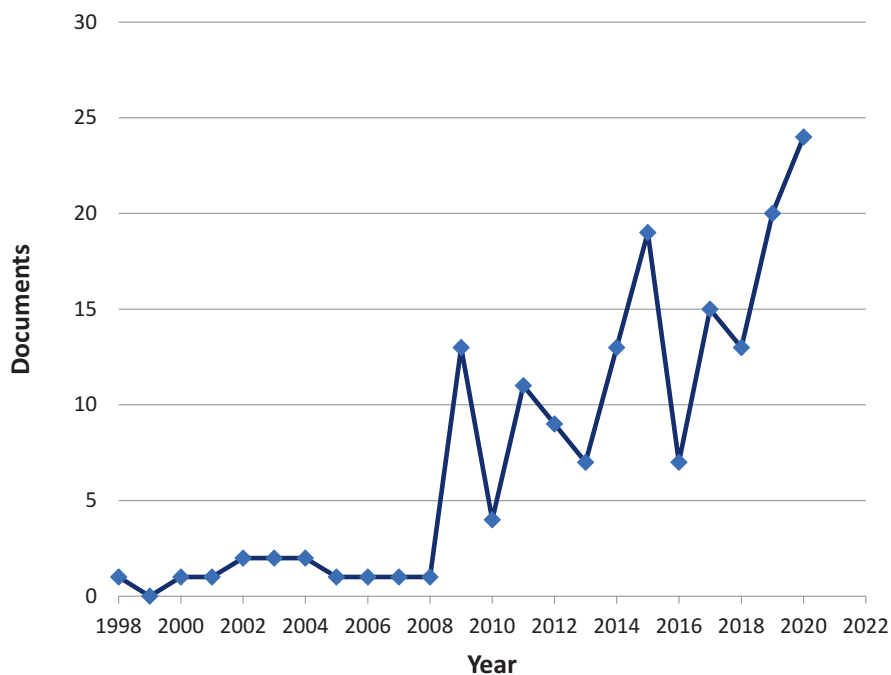
Biological activity has also been attributed to constituents of the essential oil extracted from the skin, the pulp, and the fruit's seed (Zito et al., 2013). In particular, phenolic compounds, such as thymol and carvacrol, along with terpenes (*p*-cymene, limonene, linalool, *g*-terpinene, and others), fatty acids derivatives, and aldehydes, were recognized as bioactive components of the fruit volatile fraction, the seeds being the part of the fruit with the highest concentration of these compounds. Recent investigations have provided a detailed characterization of the volatile fraction of the pulp of several prickly pear cultivars grown in Spain (Andreu-Coll et al., 2020) and of the fruit juice obtained from wild plants grown in different geographical areas of Greece (Karabagias et al., 2019). In both these studies, the bioactive volatile components, along with volatiles affecting the sensory properties of the fruit/juice, were determined, thus allowing an evaluation of the potential biological activity of the different materials and a satisfactory classification of prickly pear juices according to geographical origin (Karabagias et al., 2019), when data were subjected to linear discriminant analysis (LDA).

### 3 *Opuntia* spp. and Polyphenols: Quantitative Literature Analysis

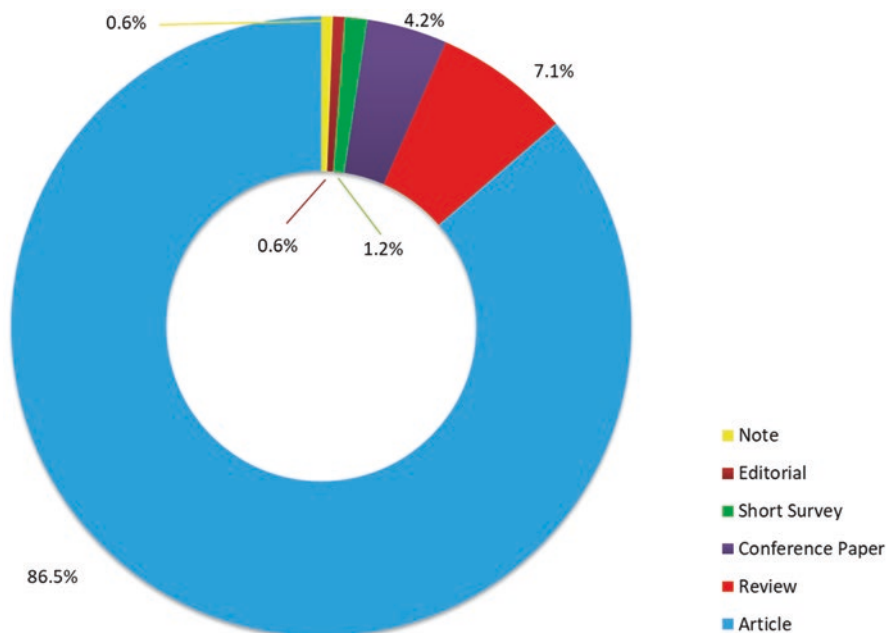
The current work provides a comprehensive overview and analysis of the polyphenol and *Opuntia* spp. relationship in literature. In December 2020, a search was conducted through the Scopus database. Bibliometric data were extracted from the

Scopus online database using the search string: “Opuntia” OR “Prickly Pear” OR “Indian fig” OR “barbary fig” OR “Cactus pear” AND “Polyphenols.” As a result, the following parameters were assessed: publication year, citation, institution, country/region, document type. The “Analyze” and “Create Citation Report” functions of the Scopus platform were utilized for the fundamental analyses. The “full records and cited references” were exported to VOSviewer software (version 1.6.11, [www.vosviewer.com](http://www.vosviewer.com)) for further bibliometric analyses. The VOSviewer software was used to analyze the titles and abstracts of publications by breaking down the paragraphs into words and phrases, associating them with the publications’ citation data, and presenting the results in the form of a bubble map (Waltman et al., 2010; Van Eck & Waltman, 2010, 2011). Default parameters were used for the analyses and visualizations. The size of a bubble represents the frequency of appearance of a term. Two bubbles are positioned more closely to each other if the terms co-appeared more often in the analyzed publications. The color represents the averaged citations per publication (CPP).

The search returned with 170 publications that were collectively cited 2620 times, with an h-index of 29 and 15.41 CPP in general. The trend of documents by year was given in Fig. 14.3, whereas the documents were distributed as reported in Fig. 14.4. The article accounts for 86.5% and Review and Conference paper for 7.1% and 4.2%, respectively.



**Fig. 14.3** Publication trends of phenolics in *Opuntia* spp. research. (Bibliometric data were extracted from the Scopus online database)



**Fig. 14.4** Distribution of documents by type. (Bibliometric data were extracted from the Scopus online database)

Figures 14.5 and 14.6 show, respectively, the most productive countries and institutions. Regarding countries/regions, the most productive was Mexico ( $n = 29$ ,  $CPP = 10.55$ ), followed by Italy ( $n = 25$ ,  $CPP = 10.16$ ), and Tunisia ( $n = 20$ ,  $CPP = 6.9$ ). It is also worth mentioning the high  $CPP$  of the works published by the United States ( $n = 9$ ,  $CPP = 25$ ) (data not shown). The most productive institution was Università degli Studi di Catania. All institutions have at least five or more publications. A total of 141 terms come from quantitative literature research on 170 publications, and they are visualized as a term map (Fig. 14.7).

#### 4 Bioactive Compounds, *Opuntia* spp., and Database: A Current State of Art

Nowadays, the need for the categorization of biological compounds is emerging. Databases can be defined as a structured system that can produce and collect data, information, and documentation, as a tool for rapid search, retrieval, and storage by a computer with several data-processing operations (Encyclopedia Britannica; Sofroniou, 2018).

The development of specialized and dedicated databases of compounds with nutritional and nutraceutical character (Durazzo et al., 2018), at both national and



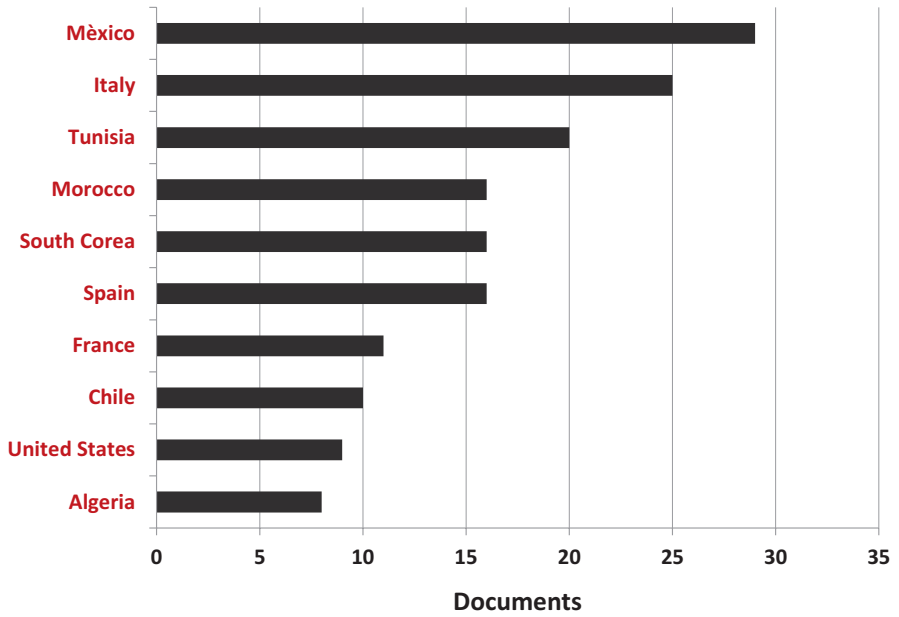


Fig. 14.5 Most productive countries. (Bibliometric data were extracted from the Scopus online database)

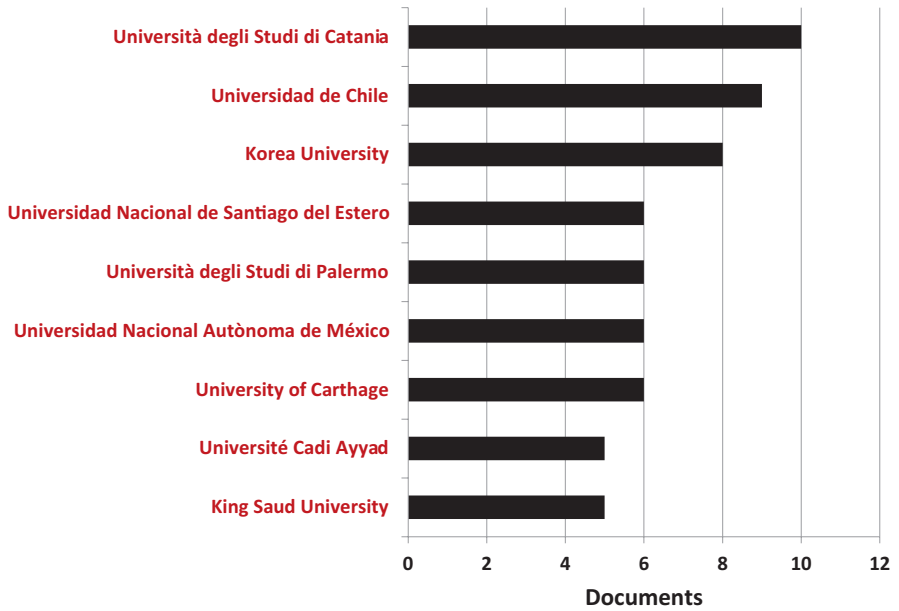
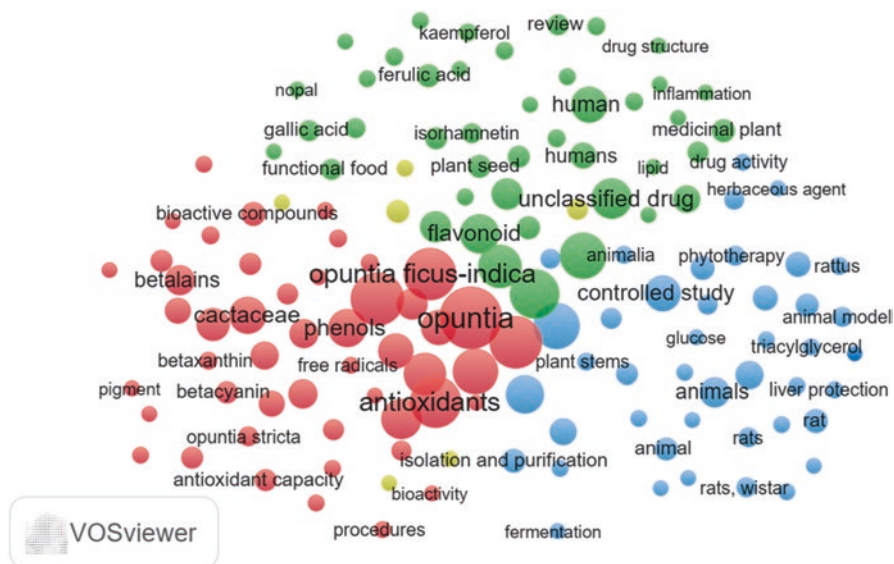


Fig. 14.6 Most productive institutions. (Bibliometric data were extracted from the Scopus online database)



**Fig. 14.7** Term map for relationship of polyphenols and *Opuntia* spp. Bubble size represents the number of publications. Bubble colour represents the citations per publication (CPP). Two bubbles are closer to each other if the terms co-appeared more frequently. (Bibliometric data were extracted from the Scopus online database and elaborated by VOSviewer software)

European/International level, is a great challenge to better investigate the relationship between food, nutrition, health, and environment, besides the great interest of researchers in all bioactive components present in food, and the increase request of managing data and organize them in the database system. Specialized databases will be a useful tool in nutrition-related studies, i.e., dietary intake assessment studies, exposure studies, and application in other fields.

As marked by Scalbert et al. (2011), in the development of a comprehensive harmonized databases, some limitations should be considered, such as the diversity of the chemical compounds, the numerous dietary sources, variability in content from a source to another source, and by the different extraction procedures as well as the analytical techniques and methodologies. Moreover, additional factors that could occur in some cases should be taken into account, i.e., the lack of appropriate analytical methods, and that only a few compounds within a class are investigated.

In FoodExplorer, an innovative interface for food composition data, that allows members to simultaneously search information from most EU Member States, as well as Canada, USA, New Zealand, and Japan, searching as digit *Prickly Pear* and selecting all 39 Databanks, we found 19 records (Finglas et al., 2014; FoodExplore website).

Moving to bioactive compounds scenario, the eBASIS database (Kiely et al., 2010; Plumb et al., 2017), considered as the first EU harmonized food composition database, combined composition data and biological effects of over 300 major

European plant foods of 24 compound classes (i.e., glucosinolates, polyphenols, isoflavones, phytosterols, glycoalkaloids, and xanthine alkaloids). EuroFIR eBASIS resource is based on a compilation work of experts that critically evaluated data extracted from peer-reviewed literature as raw data. Concerning prickly pear, in eBASIS, six data points were present for extractable and non-extractable polyphenols content (eBASIS website).

Besides the relevance of dietary supplements in the evaluation of total dietary intake, we take into account also Dietary Supplement Label Database (Dwyer et al., 2014; DSLD website), which was launched in 2013 by the Academy of Nutrition and Dietetics in the United States. This contains label information (brand name, ingredients, amount per serving, and manufacturer contact information) on more than 71,000 dietary supplements present and consumed in the US marketplace (Dwyer et al., 2014, 2018). By searching in DSLD (DSLD website) by-product/brand name and typing “prickly pear” as a keyword, research has identified four products.

## 5 Conclusion

This chapter gives an updated shot of the occurrence of *Opuntia* spp. in food and marks the importance of existing databases as a useful tool in nutrition-related studies, i.e., dietary intake assessment and exposure studies, besides the increasing attention towards the standardization and need of food categorization and classification. The overview presented in the current study should help readers better understand the relationship between polyphenols and *Opuntia* spp., identifying potential research directions, and conducting more in-depth literature searches of chemicals/chemical classes of interest. Although *Opuntia* spp. carries biologically active compounds with therapeutic potential, more detailed analyses are needed to elucidate the structure of potentially new and unknown compounds to optimize and identify bioactive chemicals in extracts from different plant parts for practical purposes in the food and medical industries.

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# Chapter 15

## Profile and Biological Properties of the Main Phenolic Compounds in Cactus Pear (*Opuntia* spp.)



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**Abstract** Natural plant-derived phenolic extract recently received much attention in the food industry to obtain bioactive compounds for food and pharmaceutical industries. These compounds are of considerable interest due to their medicinal and nutrition properties. In this line, cactus pear (*Opuntia* spp.) is considered for its potentially active nutrients and its multifunctional properties to produce health-promoting food supplements. These beneficial effects are related to its potential bioactive compounds like phenolic acids and flavonoids possessing antioxidant traits. As secondary metabolites, phenolic compounds can mitigate oxidative damage caused by cancer, cardiovascular diseases, and diabetes. The phenolic structure contains an aromatic ring with one or more hydroxyl groups that cause their therapeutic activity. Two classes of phenolic compounds are extensively investigated in the plants, including hydroxybenzoic acid and hydroxycinnamic acid. However, phenylpropanoid serves as a precursor for synthesizing the other phenolic compounds. Several phenolic compounds were found to display different health benefits, including antioxidant, anti-inflammatory, and anticancer activities. Phenolic acids and flavonoids from the genus *Opuntia* have been recognized as antioxidants. Phenolic acids such as vanillic, ferulic, *p*-coumaric, *p*-hydroxybenzoic, syringic, protocatechuic, caffeic, salicylic, gallic, and sinapic, as well as flavonoids such as rutin, isoquercitrin, kaempferol, and narcissi are found in plants from the genus

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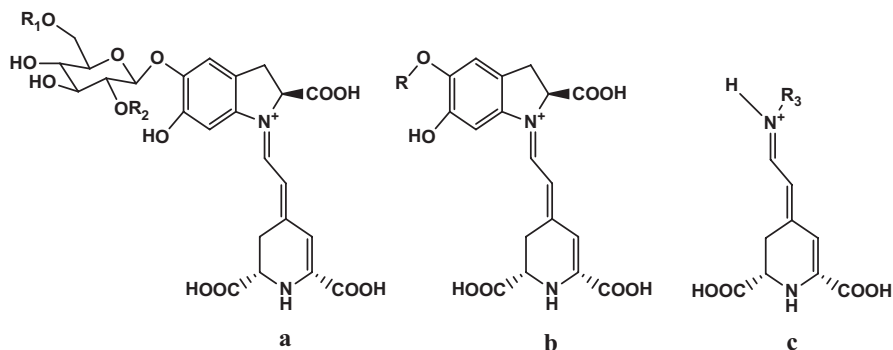
*Opuntia*. The antioxidant properties of phenolic compounds in *Opuntia* spp. plants make them an essential product for preventing human health against degenerative diseases such as cancer, diabetes, hypercholesterolemia, arteriosclerosis, cardiovascular and gastric diseases. Nowadays, the food and agricultural processing include phenolic compounds as an essential part of the human diet. This chapter discusses the medicinal importance of phenolic compounds in *Opuntia* spp. as a natural antioxidant. The article highlights the extractions methods and manufacturing processing, including backing, cooking, and frying, in the food and beverage industry and their effects on these compounds' stability and activity. This information would help identify *Opuntia* species rich in phenolics and their therapeutic properties. Besides, the chemical structure, properties, and health benefits of phenolic compounds of cactus pear fruit and their influences in agro-industrial wastes as natural sources of antioxidants with application in food industries have been reviewed.

**Keywords** Cactus · Phenolics · Antioxidants · Food chemistry · Wild food · Health · Betalain · Betacyanin · Betaxanthin

## 1 Plant-Derived Phytochemicals

Natural plant-derived bioactive compounds have been extensively studied worldwide. These compounds have been implicated in several industrial and pharmaceutical aspects (Nacz & Shahidi, 2004). Many studies recommended that plant-based functional food could reduce the risk of diseases based on oxidative stress damage (Cossu et al., 2012; Valtuena et al., 2008). Plant-derived antioxidants can be categorized under several chemical groups, including carotenoids, phenolic compounds, and polyphenolic compounds. These compounds showed a potential antioxidant activity in human health (Butera et al., 2002). There is no specific plant part contain the main content of these compounds. However, the part exposed to the harsh environmental condition could contain the highest amount where these compounds implicated in protecting plants against biotic and abiotic stresses. Some researchers suggest that the main content of antioxidants in plants is in the peel of the fruit (Yeddes et al., 2013). The natural antioxidants' role in age-dependent disease reduction and inhibition of the oxidation on food has been reported. Natural antioxidants have lower toxicity than synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene (Abou-Ellella & Ali, 2014; Andersen & Markham, 2005; Delgado-Vargas et al., 2000).





**Fig. 15.1** Chemical structure of betacyanins (a, b) and betaxanthins (c)

## 2 Cactus Pear Fruit

Due to its nutritional and health-promoting benefits, the cactus pear has attracted researchers. The prickly pear or cactus-pear are xerophytic plants belong to the new family of Opuntiaceae Desv. (syn. Cactaceae Juss.). There are 26–160 genera, and 1500–2000 species belong to this family. This plant's distribution is mainly in Mexico and in North and South America (Stintzing & Carle, 2004), and the typical genus is *Opuntia* Mill. (Reddy et al., 2005). The cactus pear plants are included in the genus *Opuntia* and characterized by cladodes, which are a kind of jointed stems. Their leaves are often absent and replaced by spines and barbed hairs (glochids) carried by areoles (axillary nodes). Usually, more than 25 flowers grow on the upper half of the cladode, and around 90–95% of flowers can transform into the fruits (Fig. 15.1) (Azeredo, 2009).

It has demonstrated that cactus pear fruit enriches bioactive antioxidants (betalains, ascorbic acid, and polyphenols) compounds (Stintzing et al., 2004). Several pharmaceutical and biochemistry investigations showed that cactus pear fruit extracts displayed antiulcerogenic, antioxidant, anticancer, neuroprotective, hepatoprotective, and antiproliferative activities (Wybraniec et al., 2007). Because of their high contents of betalains, betacyanins, and betaxanthins compounds, cactus pears have been shown coloring potential as food additives source (López et al., 2011). Moreover, intensive studies have highlighted the importance of cactus pear-derived compounds used in nutrition, health, and disease (Aksoy et al., 2013).

## 3 Chemical Composition

Cactus fruits' chemical composition is affected by the physiological stage of flowering and ripening (Table 15.1). Consequently, cactus fruits' chemical composition is varied in the types and ranges in pulp and skin. Pulp yield is an essential factor to

**Table 15.1** Chemical composition of cactus pear fruit pulps (%)

Parameter	Purple fruit	Orange fruit
Moisture	85.98	85.1
Protein	0.38	0.82
Fat	0.02	–
Fiber	0.05	–
Ash	0.32	0.26
Total sugar	13.2	14.8
$\beta$ -carotene	–	2.28
Vitamin C	20	24.1
Betainin	100	–

consider for processing, and studies have shown that the quantity of peel varies according to the zone of cultivation (Sultana et al., 2007). Carbohydrate contents showed that the pulp contains more glucose than in the skin, while the skin has more amount of saccharose than the pulp (Rodriguez-Felix & Villegas-Ochoa, 1998). Cactus fruits were also enriched in their contents of minerals such as potassium, magnesium, and calcium in both pulp and skin. The fruits, pulp, and skin of prickly pear are rich in their contents of fatty acids. Linolenic, oleic, and palmitic acids and high levels of *omega*-6 linoleic acid were reported (Ennouri et al., 2006).

The fruit, particularly its skin, is rich in vitamin E, and cactus pear fruits contain a higher amount of vitamin C compared to other common fruits like apple, banana, or grape (Ramadan & Morsel, 2003). Other vitamins were detected in a trace amount, such as vitamin K1, reflecting cactus pear fruits' high antioxidant potential activity.

## 4 Bioactive Components in Cactus Pear Fruit

### 4.1 Phenolics

Phenolic compounds are a group of secondary metabolites derived from plants and algae. They are categories into several sub-groups such as simple phenols, phenolic acids (derivatives of benzoic and cinnamic acids), coumarins, flavonoids, and stilbenes, as well as condensed, and hydrolysates tannins and lignins (Sultana et al., 2007). Betalain was the most relevant phenolic compound among all detected phenolic compounds reported in the prickly pear fruits. Betalain is the precursor of generating most of the essential phenolic compounds for both medicinal and nutritional uses. Betalain was higher in red prickly pear fruits than white and orange fruit. The total betalain contents were ranged from 81.5 to 92.0 mg 100 g<sup>-1</sup> FW. Betalains and their metabolic derivatives are responsible for the fruit colors in many species and varieties of *Opuntia* (Javanmardi et al., 2003). In this regard, betalains derivatives are represented in betacyanins and or betaxanthins compounds. Betacyanins are responsible for red-purple fruits, while betaxanthins are responsible for the orange-yellow color of the pulp and rind of fruits (Yıldırım et al., 2000).

These pigments' high content is mainly distributed at the pulp and peel of these varieties, making them good sources of natural pigments for use in the food industry as food colorant.

The biological effects of phenolic compounds have been attracting the attention of researchers in recent decades. Prickly pear fruit was examined for their phenolic contents by several investigations. Studies indicated that prickly pear fruit is an excellent source of free phenolic acids (Oniszczyk et al., 2020). Phenolic acids are the type of phenolic compounds that coupled benzoic acid derivatives. In the fruit aqueous extracts (water, methanol, and ethanol), the total contents of polyphenol reached to 50.94 mg GAE/g dry matter (Ferreira et al., 2007). Quantitative analysis of phenolic acids carried out by high-performance liquid chromatography, coupled with mass spectrometry (HPLC-ESI-MS/MS), showed up to 14 phenolic acids in the prickly fruit. Phenolic compounds represented in prickly pear fruit mostly are benzoic acid derivatives: protocatechuic, syringic, 4-OH-benzoic, vanillic, gentisic, salicylic, and cinnamic acid derivatives: caffeic, *trans*-sinapic, *cis*-sinapic, *p*-coumaric, ferulic, isoferulic, *m*-coumaric, and 3,4-dimethoxycinnamic. Isoferulic acid is dominant in the prickly pear fruit (Kapadia et al., 1996). Phenolic compounds isolated from prickly pear fruit showed important health benefits due to their antioxidant, anticancer, antibacterial, and anti-inflammatory properties.

## 4.2 *Betalains*

Betalain is the procurer of betaxanthins and betacyanins derivatives in prickly pear fruit. Betalain's concentration was higher in red prickly pear fruits than in white and orange fruits, and more concentrations reported in the peel than pulp. The total betalain levels of 92 mg 100 g<sup>-1</sup> FW in the prickly pear fruits' pulp have been reported (Sreekanth et al., 2007). In food manufacture, isolated betaxanthins and betacyanins derived from betalains have a spared uses as natural food yellow and red colorant (Fig. 15.1). These pigments are used as food coloring agents in various food products, wherein the pure yield of extracted pigments was around 1 mg/1 g dry weight. Moreover, these pigments have shown a potential antioxidant activity when compared to ascorbic acid. Cactus pear containing unique phenolic compounds that make it useful in several areas such as nutrition, traditional medicine, and industrial applications. Betanin and indicaxanthin are also involved in the increased resistance of the cells to induced oxidative stress.

## 4.3 *Flavonoids*

Due to procyanidins and phenolic acids' presence, the flavonoid contents in prickly pear fruits were lower than the phenolic content (Loro et al., 1999). Also, during the ripening of fruits, some of their flavonoids contents converted into phenolic

compounds (Kanner et al., 2001). These metabolites are also crucial as they have antioxidant, anti-inflammatory, and anticancer properties (Seeram et al., 2004). Among the advantages of cultivating *Opuntia* fruits are antioxidant compounds and the high content of stable pigments (betalains) in the pulp and peel of some varieties (Rodriguez et al., 2002). Flavonoids separated and identified from prickly pear fruit has a very high potential as pharmaceutical agents.

## 5 Biological Properties of Prickly Pear Fruit

### 5.1 Antioxidant Activities

The antioxidative action is one of the many mechanisms that exert the beneficial health properties of plants (Andres-Lacueva & Zamora-Ros, 2010). The nutritional and health properties of cactus pear fruit are associated with their content of phenolic compounds. Prickly pear fruit extracts showed an effective natural antioxidant in both the lipid and aquatic environments. The antioxidant properties of cactus pear were referred to as the presence of natural betalains (betanin and betaxanthin) (Kuti, 2004). Several studies reported the beneficial effect of colorless phenolics and betalains (Butera et al., 2002). The antioxidant potential of *Opuntia* fruit can neutralize reactive oxygen species such as singlet oxygen, hydrogen peroxide lipid peroxidation. *Opuntia* fruit's antioxidant capacity was determined by several antioxidant assays dependent factors such as the reactivity toward radicals, and the distribution, localization, and fate of antioxidant-derived radicals in interaction with other antioxidants (Kuti, 1992). Most of the antioxidant capacity associated with fruits is exerted by ascorbic acid and phenolic compounds and a mixture of purple-red betacyanin and yellow-orange betaxanthin pigments (Kuti, 1992). In a comparison between different studies on antioxidant capacity, prickly pear fruit showed that the ability to scavenge free radicals of cactus fruits is higher than pears, apples, tomatoes, bananas, white grapes and is comparable to red grapes, pink grapefruit, and red-orange (Aires et al., 2004).

### 5.2 Anticancer Activities

Studies reported that the cactus pear fruit extract displayed antiproliferation effect on various human cancer cells, including cervical, ovarian, and bladder cancer cell lines. Also, extracts have a potential activity to suppresses tumor growth in the *in vivo*-nude mice ovarian cancer animal model (AOCS, 1990). These studies recommended various doses as anticancer agent. However, no cytotoxic effect was observed when cactus extracts were administrated by intra-peritoneal into mice model animals. More importantly, tumor growth inhibition was comparable to the

synthetic retinoid N-(4-hydroxyphenyl) retinamide (4-HPR), which is used as a chemo-preventive agent in ovarian cancer chemoprevention (Meda et al., 2005). The mode of action of the antiproliferation properties of cactus pear extracts has been reported (Polshettiwar et al., 2007). They found that it is associated with apoptosis induction and cell cycle arrest at the G1-phase. Additionally, increases in the levels of tumor suppressor P53, Bax, AIF, cytochrome C was reported. The constituents and mechanism of action by cactus pear extract are not yet well explicated. Further investigations are needed to identify the potential active component(s) and the respective underlying mechanisms (Ravichandran et al., 2013). The cactus pear extracts showed anticancer activity against three different types of human cancer cells ovarian, bladder, and cervical. In this study, cervical cancer cells were the most sensitive than ovarian and bladder cancer cells. They found that 1% cactus pear fruit extract inhibited 40–60% of cervical cancer cells (Zou et al., 2005).

### 5.3 Antiviral Effects

The administration of a cactus fruit extract to mice, horses, and humans showed an inhibition of the intracellular and extracellular replication of a different DNA and RNA virus. Furthermore, the antiviral potential of the cactus extract against Herpes simplex virus Type 2, Equine herpes virus, pseudorabies virus, influenza virus, respiratory syncytial disease virus, and HIV-1 was reported. However, the mechanism of action and the active inhibitory key component(s) of the cactus extract were not investigated. No further study dealt with this specific topic (Lopez-Velez et al., 2003).

### 5.4 Anti-inflammatory Effects

Studies have identified  $\beta$ -sitosterol as an anti-inflammatory agent of the genus *Opuntia* using the fruit extract from *Opuntia dillenii* (Tesoriere et al., 2004). In cactus pear fruit, betanin and betaxanthin stimulated an inhibitory effect on the chlorination activity of myeloperoxidase at neutral rather than at pH 5 (Collazo-Siques et al., 2003). The specific particularities of cactus pear make it useful in several areas including nutrition, traditional medicine, and further industrial applications. The betanin content, indicaxanthin, betaxanthin, and betacyanin are the main factors in the health benefits and pharmaceutical activity and increased the cells' resistance to induced oxidative.

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## Chapter 16

# *Opuntia* spp. Essential Oils



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**Abstract** The cactus *Opuntia* sp. belonging to the *Cactaceae* family is a xerophyte with about 200–300 species, generally growing in arid and semi-arid zones. *Opuntia* sp. species exhibit a wide distribution in different countries due to their high ecological adaptivity and extraordinary genetic variability. These species are cultivated in countries like Argentina, Mexico, Israel, Brazil, Tunisia, Italy, and China. Fruits and stems are preferred to use for consumption as food. *Opuntia* species possess several biological activities due to their phytochemical composition, including phenolic compounds, colour pigments, and essential oil. The plant's functional properties make it beneficial to produce various food products such as juices, beverages, sweets, and jams. Essential oils have been used for their positive health effects on some diseases since ancient times. The oil of *Opuntia* spp. has different health properties such as antioxidant, and antimicrobial activities. One member of *Opuntia* spp. is rich in thymol (up to 45%), which exhibits strong antioxidant and antimicrobial activities. The other species, *Opuntia ficus-indica*, presents a varying range of volatile components depending on the plant's part. The plant's red skin contains high hydrocarbons, while yellow skin is rich in aldehydes, ketones, and hydrocarbons. The number of terpenes is higher in the red pulp; however, the yellow pulp contains fatty acids and derivatives as major components. Hydrocarbons are the main components of red seeds, while yellow seeds contain mainly fatty acids and their derivatives. A Tunisian cactus pear (*Opuntia ficus indica* L.) flower contains a low level of essential oil (0.01%), including benzenacetatealdehyde, D-3-carene, hexanol, and  $\alpha$ -pinene as the major volatile components.

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## 1 Introduction

The *Opuntia* sp. belongs to the *Cactaceae* family. *Opuntia* grows in arid and semi-arid regions and temperate or tropical environments (Peña-Valdivia et al., 2008). The *Opuntia* genus consists of more than 1500 species distributed in various countries from Canada to Argentina. They are widely distributed in Mexico, an important center of high diversity (Reyes-Agüero et al., 2006). The major *Opuntia* plant producer country is Mexico, while other commercial cultivation areas are in Spain, Italy, Brazil, Chile, Argentina, and the USA (Kalegowda et al., 2015).

The plant is also called prickly pears due to their edible fruit parts (Piga, 2004). There are many other species of *Opuntia* that have potentially beneficial effects. For instance, *O. dillenii* (Ker-Gawl) Haw. is another important species for its health properties and is commonly present in India's arid southern parts (Kalegowda et al., 2017).

The cactus plant can be divided into different parts: root, the vegetative part, fruit, and flower. Especially, fruit and vegetative parts (cladodes) are valuable parts of cactus. Cactus plant is generally evaluated for fruit production and forage in some countries (Stintzing & Carle, 2005; Ayadi et al., 2009). The fruits are used to produce various food products such as juices, marmalades, gels, jam, sweetener, canned fruit, frozen fruit, juice concentrate, juice powder, and alcoholic drinks (Saenz, 2000; Feugang et al., 2006). Beside fruits, the cladodes also serve as sources for food production. Saenz (2000) studies and Feugang et al. (2006) demonstrated that cladodes are used in pickles, brine, candy, marmalades, jam, flour, sauce, cooked vegetable, alcohol, and edible coatings. Besides, by-products from fruit and cladodes serve as sources for dietary fiber production (Ayadi et al., 2009; Bensadón et al., 2010), oil production (Ramadan & Mörsel, 2003), natural antioxidants production (Cardador-Martínez et al., 2011), and pigment production (Melgar et al., 2017).

Nowadays, the scientific interest in *Opuntia* spp. has increased mainly because of its valuable chemical composition. The chemical composition of *Opuntia* changes depending on the variety, location, and part of the plant. The fruit pulp of *Opuntia ficus-indica* mainly contains total solids (11.2%), ash (0.06%), protein (0.21%), total sugars (9.83%) (de Souza et al., 2015). The fruit of *O. dillenii* is rich in fiber (9.49%), lipids (0.71%), ash (0.43%) compared with the fruit of *O. ficus indica*. However, *O. ficus indica* contains higher amounts of proteins (0.90%) than *O. dillenii* (Díaz Medina et al., 2007). The water content of pulp and skin (94% and 90%, respectively) of *Opuntia ficus-indica* was higher than the seeds (18%). The seed part of this plant was rich in protein and lipid (4.48% and 3.66%, respectively), while pulp and skin parts had lower protein and lipids (Salim et al., 2009). Location was reported to significantly affect the seed oil, some sugars (saccharose, and raffinose),

total phenolics,  $\beta$ -carotene, ascorbic acid values of *Opuntia ficus-indica* fruits (Belviranlı et al., 2019). The seeds are rich in a fixed oil (7.3–9.3%). The oils of *Opuntia ficus-indica* seeds contained a high level of unsaturated fatty acids, especially linoleic acid (58.7–63.1%), followed by oleic acid (15.2–24.3%) (Chougui et al., 2013).

In addition to major compounds, *Opuntia* spp. contain a wide variety of minor compounds. Most of these compounds have remarkable healing effects versus many human diseases. (all-E)-Lutein is the major carotenoid representing 71–72% of the total carotenoids in the fruit tissues of *Opuntia ficus-indica* (Butera et al., 2002). Pericarp part of *Opuntia joconostle* contains high amounts of phenolic compounds and total flavonoids (2.07 mg gallic acid equivalents (GAE)/g fresh weight (FW) and 0.46 mg (+)-catechin equivalents (CE)/g FW, respectively) compared that of endocarp and mesocarp. In the fruit extracts, protocatechuic, 4-hydroxybenzoic, caffeic, vanillic, and syringic acids, as well as rutin, and quercetin are identified as phenolic compounds. The betacyanin, which is responsible for the color of the fruit, has been detected in higher amounts in endocarp (23.0 mg betanin equivalents/100 g fresh weight) (Osorio-Esquivel et al., 2011). Other than these mentioned minor compounds, the essential oil is the most crucial part of *Opuntia* spp. Essential oil contents and compositions depend on variety, cultivar, part of the plant, and location. The essential oil contents of three varieties of *Opuntia* (*O. littoralis*, *O. ficus-indica*, and *O. prolifera*) were reported to vary between 0.0012% and 0.28% (Wright & Setzer, 2014). This chapter discussed the chemical composition of essential oil, the effect of different factors on essential oil composition and biological properties of essential oils, as well as the plant itself.

## 2 Essential Oil Composition

Essential oils are the volatile part of the plant, which comprised different constituents. Essential oils are generally liquid and colorless at room temperature. They are widely used in the perfumery, cosmetics industry, and aromatherapy. Essential oil composition varies depending on variety, location, environmental conditions, and production methods (Vigan, 2010; Dhifi et al., 2016).

### 2.1 Species

Essential oil is a minor part of *Opuntia* spp. The chemical compositions of essential oils from different *Opuntia* species are presented in Table 16.1. Moosazadeh et al. (2014) identified 19 compounds in the fruits' essential oils from *Opuntia stricta*. The main identified compounds were thymol (42.7%) and n-octane (18.6%). A study by Wright and Setzer (2014) demonstrated that *O. littoralis* oil included

**Table 16.1** Chemical composition of *Opuntia* spp. essential oils

	<i>Opuntia stricta</i> <sup>a</sup>	<i>Opuntia littoralis</i> <sup>b</sup>	<i>Opuntia ficus-indica</i> <sup>b</sup>	<i>Opuntia proliferata</i> <sup>b</sup>
<i>n</i> -octane	18.6	–	–	
Thymol	42.7	–	–	
β-Caryophyllene	9.2	–	–	
Caryophyllene oxide	3.8	–	–	
Epi-α-Cadinol	4.8	–	–	
Palmitic acid	4.3	4.4	12.7	
<i>cis</i> -linalool oxide	–	10.8	–	
<i>trans</i> -linalool oxide	–	8.8	–	
2-Furaldehyde	–	3.8	–	
2-Hexanol	–	3.5	–	
Lauric acid	–	–	10.5	
Myristic acid	–	–	4.2	
(E)-phytol	–	–	8.0	
Linoleic acid	–	–	22.3	
(9Z)-Tricosene	–	–	6.7	
Heptadecane	–	–	–	19.2
Pentadecane	–	–	–	5.1
Dodecanoic acid	–	–	–	5.3
Hexadecane	–	–	–	3.8

Adapted from <sup>a</sup>Moosazadeh et al. (2014), <sup>b</sup>Wright and Setzer (2014)

Only the percentage values of above 3.5% are given

mainly terpenoid-derived compounds (47.2%) and fatty-acid derived compounds (29.1%). The main identified compounds in *O. littoralis* oil were *cis*-linalool oxide (10.8%) and *trans*-linalool oxide (8.8%). Besides, the major chemical group was fatty acids in the oil from another *Opuntia* sp., *O. ficus-indica*, with linoleic acid (22.3%), palmitic acid (12.7%), and lauric acid (10.5%). In the same study, *O. proliferata* oil was made up of alkanes (47.1%), and the principal compound was heptadecane (19.2%).

## 2.2 Plant Part

The essential oil composition of *Opuntia* differs depending on the part of the plant (Table 16.2). Zito et al. (2013) analyzed essential oil compositions of different parts (skin, pulp, seed) from the red (Sanguigna cultivar) and yellow (Surfarina cultivar) fruits of Sicilian *O. ficus-indica*. A total of 41 compounds were identified in *O. ficus-indica* fruits' oil, while a different number of components were identified in different parts. Considering the fruit's skin, hydrocarbons (43.5%) constituted most chemical groups in oils from red skin. In comparison, the oils from yellow skin

Table 16.2 Major compounds in the essential oil from different parts of *Opuntia* spp.

	<i>O. ficus-indica</i> <sup>a</sup>						<i>Opuntia lindheimeri</i> var. <i>linguiformis</i> <sup>b</sup>						<i>Opuntia macrorhiza</i> <sup>b</sup>		<i>Opuntia microdasys</i> <sup>b</sup>		<i>O. ficus-indica</i> <sup>c</sup>
	Red			Yellow			Leaves	Flowers	Fruits	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers		
	Skin	Pulp	Seed	Skin	Pulp	Seed											
Heptacosane	11.9	6.7	7.4	7.8	-	-	-	-	-	-	-	-	-	-	-	-	
Pentacosane	8.7	5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Tricosane	8.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Carvacrol	7.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
(E,E)- $\alpha$ -Farnesene	6.5	-	-	5.7	-	-	-	-	-	-	-	-	-	-	-	-	
<i>p</i> -Vinylguaiaicol	5.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Hexahydrofarnesylacetone	5.4	-	-	19.1	-	-	-	-	-	-	-	-	-	-	-	-	
Nonacosane	5.2	-	9.6	-	-	-	-	-	-	-	-	-	-	-	-	-	
$\gamma$ -Dodecalactone	-	-	-	6.7	-	-	-	-	-	-	-	-	-	-	-	-	
$\gamma$ -2-Dodecenolactone	-	-	-	5.6	-	-	-	-	-	-	-	-	-	-	-	-	
Limonene	-	10.4	-	-	5.5	-	-	-	-	-	-	-	-	-	-	-	
<i>p</i> -cymene	-	9.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Carvacrol methyl ether	-	5.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2,4,6-Trimethylbenzaldehyde	-	5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Squalene	-	27.2	5.2	-	5.3	-	-	-	-	-	-	-	-	-	-	-	
Hexadecanoic acid	-	-	28.5	-	33.0	65.7	8.50	16.70	17.33	-	-	-	7.20	13.13	-	-	
Octacosane	-	-	5.6	-	-	-	-	-	-	-	-	-	-	-	-	-	
Triacotane	-	-	7.7	-	-	-	-	-	-	-	-	-	-	-	-	-	
Tetradecanoic acid	-	-	-	-	-	-	13.57	-	-	-	-	-	-	-	-	-	
Hexanol	-	-	-	-	-	-	-	-	8.55	-	-	-	-	-	-	9.57	

(continued)

Table 16.2 (continued)

	<i>O. ficus-indica</i> <sup>a</sup>						<i>Opuntia lindheimeri</i> var. <i>linguiformis</i> <sup>b</sup>						<i>Opuntia macrorhiza</i> <sup>b</sup>		<i>Opuntia microdasys</i> <sup>b</sup>	<i>O. ficus-indica</i> <sup>c</sup>
	Red			Yellow			Leaves	Flowers	Fruits	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers	
	Skin	Pulp	Seed	Skin	Pulp	Seed										
Nonanoic acid	-	-	-	-	-	-	7.16	-	-	-	-	10.57	7.56	-	-	
Butyl tetradecanoate	-	-	-	-	-	-	8.05	21.47	8.18	-	-	21.41	5.91	-	-	
(E)-5-Butyldiene phthalide	-	-	-	-	-	-	13.17	6.92	15.77	14.02	-	-	-	-	-	
D-3-carene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11.38	
$\alpha$ -Pinene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.49	
Benzenacetatealdehyde	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26.31	

Adapted from <sup>a</sup>Zito et al. (2013), <sup>b</sup>Bergaoui et al. (2007), <sup>c</sup>Ouerghemmi et al. (2017)  
 Only the percentage values of above 5% are given

mostly included aldehydes and ketones (21.8%), and hydrocarbons (21.4%) as major chemical groups. As for pulps, terpenes (49.7%) were the major groups in the oil from the red pulp, while the yellow pulp contained mainly fatty acids and derivatives (56.9%) and terpenes (16.7%). Regarding the seeds, the oil from red seeds was rich in hydrocarbons (38.5%) and fatty acids and derivatives (31.9%), while the oil of yellow seeds mostly included fatty acids and derivatives (68.9%). When analyzed by individual components in oils from the Sanguigna cultivar (red), heptacosane (skin), squalene (pulp), and hexadecanoic acid (seed) were identified as major compounds. In contrast, the main compounds in the oil of Surfarina cultivar (yellow) were hexahydrofarnesylacetone (skin) and hexadecanoic acid (pulp and seeds).

Another study by Bergaoui et al. (2007) revealed that the leaves of *Opuntia lindheimeri* var. *linguiformis* were rich in carboxylic acids (30.7%) and esters (22.7%). However, esters were the major chemical groups in which the flowers' volatile extracts had an ester content of 34.8%, while the fruits had 24.9%. As for individual volatile compounds in leaves, tetradecanoic acid (13.6%) and (*E*)-5-Butyldiene phthalide (13.2%) found as major compounds, followed by hexadecanoic acid (8.5%), butyl tetradecanoate (8.1%), and nonanoic acid (7.2%). The major compounds found in the volatile extract obtained from the flowers were butyl tetradecanoate (21.5%) and hexadecanoic acid (16.7%), while fruits contained higher amounts of hexadecanoic acid (17.3%), and (*E*)-5-butyldiene phthalide (15.8%). The major compound was an ester, (*E*)-5-butyldiene phthalide (14.0%) in the volatile fraction from the leaves of *Opuntia macrorhiza*. The plant's flower part contained a high concentration of butyl tetradecanoate (21.1%) and nonanoic acid (10.6%). (*E*)-3-Butyldiene phthalide content was 21.4% in the volatiles of flowers in *Opuntia microdasys*.

The volatiles of skins and pulps of *O. microdasys* and *O. macrorhiza* were profiled using a modern extraction method, namely HS-SPME. It was reported that the major compound was camphor in *O. microdasys* skins (49%) and pulps (26%), followed by nonanal (16.4%) and 1-nonanol (22%). As for *O. macrorhiza*, the major volatile compound was camphor in the fruit skin part (39%), while the predominant volatile of fruit pulp was ethyl acetate (54%), followed by 1-hexyl acetate (15.3%) (Chahdoura et al., 2019).

The most abundant compound in the flower essential oil from *Opuntia ficus indica* was benzenacetatealdehyde (26.3%), followed by D-3-carene (11.4%), hexanol (9.6%),  $\alpha$ -pinene (8.5%), and geranyl acetate (4.9%) (Ouerghemmi et al., 2017). Another study by De Leo et al. (2010) revealed that the main compounds were germacrene D (12.6%), followed by 1-hexanol (12.3%), n-tetradecane (9.1%), and decanal (8.2%) in the essential oil from *Opuntia ficus-indica* flowers.

### 2.3 Location

Growing area or location is another essential factor in the volatile compounds of *Opuntia* spp. In a study by Wright and Setzer (2013), the essential oil composition of *Opuntia acanthocarpa* var. *major* and *Opuntia phaeacantha* var. *discata* growing in two areas in Arizona were evaluated using a gas chromatography-mass

spectrometer. The authors revealed that octanoic acid was the major volatile (12.3%) in *O. acanthocarpa* oil from the Organ Pipe Cactus National Monument. In comparison, eupatoriocromene (with a concentration of 66.6%) constituted 71.5% of the total oil of *O. acanthocarpa* oil growing in the Arizona-Sonora Desert Museum. The major compound of the oil from *Opuntia phaeacantha* growing in the Organ Pipe Cactus National Monument was tricosane (15.5%). In comparison, *p*-vinylguaiaicol (16.2%) was found as the major compound in the oil from *Opuntia phaeacantha* growing at the Arizona-Sonora Desert Museum. The authors also revealed that these differences could be related to soil type and rainfall.

## 2.4 Extraction Technique

The extraction technique also influences the volatile compounds of *Opuntia* spp. HS-SPME technique is a new method to determine volatiles in plants. Less preparation time is spent with this technique. Also, it reduces the complexity of sample handling or complicated apparatus and is sensitive to volatiles according to classical methods (Kataoka et al., 2000; Balasubramanian & Panigrahi, 2011; Merkle et al., 2015).

Agozzino et al. (2005) determined the volatile compounds of the yellow (Surfarina cultivar), the white (Muscaredda), and the red (Sanguigna) cultivars of *Opuntia ficus indica* using the HS-SPME technique. Alcohol was the major group of compounds identified in all samples. The major compound in volatiles of the yellow and white cultivars is (*E*)-2-hexen-1-ol [15.9% and 19.5%, respectively, followed by 2-nonen-1-ol (13.9% and 16.9%, respectively)]. In the red cultivar, (*E*)-2-Nonen-1-ol was reported as the major compound with the level of 21.9%, followed by (*E*)-2-hexen-1-ol (16.3%).

Oumatou et al. (2016) investigated the volatiles of three cultivars of *O. ficus-indica* from Morocco, i.e., (a) Dellahia (a white cultivar), (b) Aissa (a red cultivar), and (c) Shoul (a yellow cultivar) using SPME technique. Aldehydes and alcohols were the major chemical groups found in the volatiles of *O. ficus-indica* fruits. The most abundant compounds in the Dellahia cultivar were (*Z*)-2-hexen-1-ol (10.8%), 2-hexanal (10.6%), and *n*-hexanol (10.3%). In the volatiles of Aissa cultivar, the main compound was (*E*)-2-nonenal with a level of 16.7%, followed by (*E*, *Z*)-2,6 nonadienal (14.4%). However, 2-hexanal was found at a higher concentration (44%) in the Shoul cultivar than other cultivars.

Farag et al. (2017) investigated the volatile compounds in the fruits from three cultivars (red 'Rose', yellow-orange 'Gialla' and greenish-white 'Bianca') of *Opuntia ficus-indica* using HS-SPME. Aldehydes/ketones and short-chain acids were the major groups of compounds, accounting for 32.4%, 29.9%, 27.6%, 26.2%, and 25.2%, 25.2% of volatiles from green, red and yellow cultivars, respectively. Nonanal was the major aldehyde found in all cultivars (4–8%). Among short-chain acids, heptanoic acid (1.46–6.06%), octanoic acid (4.61–8.74%), and nonanoic acid (3.73–11.9%) were the major compounds.



## 2.5 Maturity Stage

The maturity stage is another factor in the composition of volatile compounds. Chahdoura et al. (2016) examined the volatile compounds of *Opuntia microdasys* (Lehm.) flowers at three different stages (the vegetative stage, the full flowering stage, and the post-flowering stage) using the solid-phase microextraction (SPME) technique. Oxygenated monoterpenes were the major chemical groups found at *Opuntia* samples during the maturation of flowers. Among volatiles, another important group was esters, especially methyl and ethyl esters of hexanoic, heptanoic, octanoic, and nonanoic acids. Camphor was identified as the major volatile at all flowering stages, with a range of 40 and 48%. After camphor, ethyl nonanoate (9.1% in the vegetative stage), carvone (15.8% in the full flowering stage), and ethyl hexanoate (6.1% in the post-flowering stage) were other important volatiles in *Opuntia* samples at 3 different maturation stages.

## 2.6 Drying

The effect of drying on the volatile compounds of *Opuntia dillenii* (Ker-Gawl) Haw. was investigated by Xu et al. (2015). In the fresh samples, aldehydes (41.8%), alcohols (17.8%), ketones (13.4%), alkanes (9.2%), and esters (6.4%) were found as main classes of compounds in fresh samples. However, the predominant compounds in dry samples were aldehydes (57.78%), alkanes (11.92%), acids (5.94%), alcohols (5.43%), and ketones (2.86%). The most abundant compound in the fresh sample is nonanal (11.44%), followed by hexanal (5.853%), octanal (5.151%), and (E)-2-decenal (5.075%). In dry samples, hexanal was quite abundant (13.057%), followed by (E)-2-decenal (12.835%) and nonanal (11.336%).

## 2.7 Antimicrobial Activity

Essential oils have been widely studied among natural antimicrobials, which have been presented as a potential for using instead of using chemical antimicrobials. Essential oils exhibit a strong effect against different microorganisms, especially spoilage microorganisms, foodborne, and postharvest pathogens (Langeveld et al., 2014; Aziz & Karboune, 2018). The essential oil of *Opuntia stricta* fruit exhibited antimicrobial activity against Gram-positive bacteria (*Bacillus cereus*, *Bacillus licheniformis*, *Escherichia coli*, and *Pseudomonas aeruginosa*) and *Candida albicans* in concentrations of 20 and 40 mg/mL according to the disc-diffusion method (Moosazadeh et al., 2014).

Bergaoui et al. (2007) reported that the volatile fraction of three *Opuntia* species, namely *Opuntia lindheimeri* var. *linguiformis*, *Opuntia macrorhiza*, and *Opuntia*

*microdasys*, showed antifungal activity *in vitro* using the disc diffusion method. The study revealed that the volatile fractions of leaves (58.8 mm) and fruits (46.8 mm) of *Opuntia lindheimeri* var. *linguiformis*, leaves (61.3 mm), and flowers (61.1 mm) of *Opuntia macrorhiza*, and the leaves (48.3 mm) of *Opuntia microdasys* showed activity against *Alternaria solani*. Besides, the volatile fraction from flowers of *Opuntia lindheimeri* var. *linguiformis* flowers exhibited activity against *Fusarium oxysporum* f. sp. *niveum* with the moderate inhibition level (16.8 mm). Also, *Opuntia macrorhiza* leaves (21 mm) showed activity against *Rhizoctonia solani* at 1 mg/mL concentration.

### 3 Health Properties of *Opuntia* spp.

*Opuntia* species have been widely studied regarding their health-promoting and functional properties. Although different parts, including pulp, fruit, and juice of the prickly pear, were already used due to their therapeutic effects in folk medicine, recent scientific studies have thoroughly revealed the fruit's curative properties. One of the *Opuntia* spp., i.e., *Opuntia monacantha*, has been reported for its anti-diabetic effects in rats (Yang et al., 2008). The researchers reported the positive effects of the polysaccharides of the *Opuntia monacantha* cladode in controlling the blood glucose and high-density lipoprotein cholesterol levels of the rats. In another study by Serra et al. (2013), the antiproliferative effects of the residues of *Opuntia ficus-indica* and *Opuntia robusta* have been investigated in human colon cancer. According to their results, the examined residues from *Opuntia* spp. It can be described as good sources for such phytochemicals as betacyanin, ferulic acid, and isorhamnetin derivatives, which can stop the cell cycle and result in cancer cells' death. Betacyanins were also reported to be effective against liver damage in a study by González-Ponce et al. (2020). The researchers stated that the *Opuntia* spp. were rich in betacyanins, particularly betanin and its isomer-isobetainin. According to the researchers, the betacyanin concentrations in the extracts of *Opuntia robusta* and *Opuntia streptacantha* were 464.9 and 148.9 mg eq./L. Due to the former's higher betacyanin content, this sub-species was reported to exhibit a higher protective effect against acetaminophen (APAP) induced liver failure than the latter. The neuroprotective effect of *Opuntia ficus-indica* var. *saboten* has been reported by Dok-Go et al. (2003). According to the researchers, the oxidative damages in cortical cells could be prevented by the neuroprotective effects of the fruits and the plant's stems due to its flavonoid content mainly constituted by quercetin, (+)-dihydroquercetin, and quercetin 3-methyl ether. The authors also concluded that quercetin 3-methyl ether was the most prominent component among these flavonoids. Besides many health-protective attributes, *Opuntia* has been more studied for its antioxidant activity by various researchers (Ammar et al., 2015; Coria-Cayupán et al., 2011; Smida et al., 2017; Zeghad et al., 2019).

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# Chapter 17

## Antioxidant Activity of *Opuntia* spp.: A Review



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**Abstract** *Opuntia* spp. are well-known for their agronomical, nutritional, and medicinal values owing to their richness in bioactive phytochemicals and their derived products. *Opuntia* spp. is a good source of dietary antioxidants having a key role in preventing and treating various human chronic and degenerative diseases. The different parts of *Opuntia* spp., namely cladodes, fruits, peels, pulp, and seeds, exhibit beneficial properties, mainly resulting from their high contents in antioxidants as phenolics, vitamins, sterols, carotenoids, and betalains. To better understand the mechanism underlying the antioxidant activity of *Opuntia* spp., the present work highlighted the antioxidant activity of the phytochemicals in *Opuntia* spp. and summarized the method used to evaluate the antioxidant mechanism of action. Some suggestions for future studies were also presented.

**Keywords** Antioxidant potential · Bioactive compounds · Evaluate methods · Mechanism of action · Sterol · Carotenoid · Betalain

### Abbreviations

$^1\text{O}_2$	Singlet oxygen
ABTS	2,20-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
AChE	Acetylcholinesterase
BChE	Butyrylcholinesterase
CAT	Catalase
$\text{CO}_3^{2-}$	Carbonate
COX	Cyclooxygenase
$\text{Cu}^{2+}$	Copper ion
CUPRAC	Cupric ion-reducing antioxidant capacity
DPPH $\cdot$	2,2-Diphenyl-1-picrylhydrazyl

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Fe <sup>2+</sup>	Ferrous ion
FRAP	Ferric reducing-antioxidant power
GGT	$\gamma$ -Glutamyltranspeptidase activity
GR	Glutathione reductase
GSH	Reduced glutathione
GSHPx	Glutathione peroxidase
GSt	Glutathione-S-transferase
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HOCl	Hypochlorous acid
IL-10	Interleukin-10
IL-1 $\beta$	Interleukin-1beta
IL-6	Interleukin-6
LDL	Low-density lipoprotein
LOX	Lipoxygenase
LPO	Lipid peroxidation
LPS	Lipopolysaccharides
MPO	Myeloperoxidase
NAD	Nicotinamide adenine dinucleotide
NADP	Dinucleotide phosphate
NF- $\kappa$ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide
NOS	Nitric oxide synthase
NOX	Nitrogen oxidase
Nrf-2	Nuclear factor erythroid 2
O <sup>2-</sup>	Superoxide anion
O <sub>3</sub>	Trioxxygen
OH	Hydroxyl radical
ONOO <sup>-</sup>	Peroxynitrite
ORAC	Oxygen radical absorbance capacity
PCL	Photochemiluminescence
pH	Power of hydrogen
RNS	Reactive nitrogen species
ROO	Peroxyl radical
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TEAC	Trolox equivalent antioxidant capacity
TGF- $\beta$	Transforming growth factor-beta
TNF- $\alpha$	Tumor necrosis factor-alpha
TRAP	Total radical-trapping antioxidant parameter
UVA	Ultraviolet A
XO	Xanthine oxidase

## 1 Introduction

Antioxidant activity has become a very popular term in modern society as related to foods and health benefits. Antioxidant activity is technically known as the ability of a bioactive substance to reduce free radical and scavenge ROS (HOCl, O<sub>3</sub>, ONOO, <sup>1</sup>O<sub>2</sub>, and H<sub>2</sub>O<sub>2</sub>), inhibiting and/or restoring injuries produced by oxidation and degradation of other molecules (Roginsky & Lissi, 2005). Oxidation is a reaction promoted by enzymatic, chemical, and/or physical factors that outcomes in a loss of electrons from a given substance (Battino et al., 2002). As a result, this reaction may produce free radicals that start chain reactions and accelerate the oxidative degradation of biochemically essential molecules such as lipids, proteins, and nucleic acids, among others. Oxidative stress plays a significant role in many human diseases, including cancer and neurodegenerative disorders, justifying antioxidants' extensive use in dietary supplements for their chemoprevention (Kozarski et al., 2011).

Bioactive compounds play a pivotal role as antioxidants. They are the major antioxidant sources from plants reducing the oxidative stress-induced tissue damage due to chronic diseases. Apart from their application in the pharmaceutical industries, bioactive compounds are being used in the food industry to produce nutraceuticals, novel healthy food formulations, and animal feed supplementations (Chaudhari et al., 2017).

*Opuntia* spp. are an interesting source of bioactive compounds/products belonging to the dicotyledonous angiosperm Cactaceae family, of which about 1500 species of cactus are known (Butera et al., 2002). There are almost 300 species of the genus *Opuntia* (Yahia & Sáenz, 2011) distributed in several regions, occupying around 100,000 ha, especially in south America, the Mediterranean Basin, the Middle East, and India (Msaddak et al., 2017). *Opuntia* spp. are capable of growth in almost all climates, likely arid, temperate, and tropical climates (Lallouche et al., 2015). Additionally, they can be founded in desert zones having difficult environmental conditions. *Opuntia* spp. have been used for centuries as food resources and in traditional medicine to cure several chronic diseases such as obesity, cardiovascular and inflammatory diseases, diabetes, and gastric ulcers (Young et al., 2005). They are also used in agrochemicals, cosmetics, food industries (Barba et al., 2017), and wastewater treatments (Ben Rebah & Siddeeg, 2017). *Opuntia* spp. contain vitamins, flavonoids, coumarins, carotenoids, pectins, and other bioactive compounds (Aruwa et al., 2018). These and other phytochemical groups, derived from other natural products, bring about several health benefits such as antioxidant, anti-inflammation, antimutagenicity, anticarcinogenic, and anti-aging activities (Barba et al., 2017). To afford a comprehensive view of current studies on the antioxidant activity of *Opuntia* spp., the antioxidant components and their antioxidant activities, evaluation methods, and the mechanisms of antioxidant action of *Opuntia* spp. were systematically reviewed.



## 2 The Antioxidant Components of *Opuntia* spp. and Their Activities

### 2.1 Phenolic Compounds in *Opuntia* spp. and Their Antioxidant Activities

Generally, phenolic compounds act as the main contributors to the antioxidant potential of plant extracts. Their characterization could provide considerable benefits to individuals, mainly through inciting their use as healthy promoters. There is a consensus that many of the health benefits of several dietary phenolic compounds might be attributed to their antioxidant mechanisms in fighting chronic diseases associated with oxidative damage (Arts & Hollman, 2005).

All plant parts of *Opuntia* spp. are rich in several phenolic compounds as flavonoids and phenolic acids (Table 17.1). In the flowers of *O. dillenii*, *O. ficus indica*, and *O. microdasys*, isorhamnetin derivatives were the major compounds varying from 2230 to 22,336  $\mu\text{g/g}$  (Ahmed et al., 2005; De Leo et al., 2010; Yeddes et al., 2014; Chahdoura et al., 2014a, b; Ouerghemmi et al., 2017). Similarly, isorhamnetin derivatives (545–2860  $\mu\text{g/g}$ ) were also abundant in the whole fruits of *O. ficus-indica* and *O. engelmannii* (Salem et al., 2020). Isorhamnetin derivatives were the main compounds in *O. engelmannii* (5.37  $\mu\text{g/g}$ ) and *O. microdasys* (290  $\mu\text{g/g}$ ) fruit peels (Chahdoura et al., 2019), and *O. microdasys* (2507  $\mu\text{g/g}$ ) cladodes (Chahdoura et al., 2014a, b). For *O. robusta*, *O. rastrera*, *O. streptacantha*, and *O. undulata*, their cladodes contained a high content of isorhamnetin ranging from 99.5 to 326.9  $\mu\text{g/g}$  (Santos-Zea et al., 2011). Isorhamnetin possesses antiinflammatory activity (Kim et al., 2013, 2014), mostly through its direct antioxidant action in reducing oxidative damage (Antunes-Ricardo et al., 2015). In the study of Antunes-Ricardo et al. (2015), isorhamnetin and its derivatives, isolated from *O. ficus indica* cladodes, were found to have a strong antiinflammatory activity by inhibition of nitric oxide production in RAW264.7 cells.

In *O. macrorhiza*, the fruit peel is rich in quercetin derivatives with 1240  $\mu\text{g/g}$  (Chahdoura et al., 2019). Quercetin was the major compound (43.2–90.5  $\mu\text{g/g}$ ) in the whole fruits of *O. dillenii*, *O. lindheimeri*, *O. stricta*, *O. undulata*, and *O. streptacantha* (Kuti, 2004; Fernández-López et al., 2010; Betancourt et al., 2017). Quercetin is a natural antioxidant, producing its antioxidative actions by inhibiting lipid peroxidation through blockade of the enzyme xanthine oxidase, chelating iron, and directly scavenging hydroxyl, peroxy, and superoxide radicals (Takizawa et al., 2003). Quercetin and its derivatives (quercetin 3-methyl ether, and dihydroquercetin) were isolated from *O. ficus-indica* fruits (DoK-Go et al., 2003; Kim et al., 2016) and stem (DoK-Go et al., 2003). DoK-Go et al. (2003) reported that quercetin, quercetin 3-methyl ether, and dihydroquercetin markedly inhibited lipid peroxidation scavenged DPPH<sup>•</sup> free radicals. Among these three isolated compounds, DoK-Go et al. (2003) noted that quercetin 3-methyl ether appeared to have the most potent

**Table 17.1** Distribution and contents of phenolics in the various parts of *Opuntia* spp.

	Plant part	Main compound	Content (µg/g)	References
<i>O. ficus indica</i>	Flower	Isorhamnetin derivatives	2230	De Leo et al. (2010)
		Quercetin derivatives	1180	Yeddes et al. (2014)
		Kaempferol derivatives	410	Ouerghemmi et al. (2017)
<i>O. dillenii/O. microdasys</i>		Isorhamnetin derivatives	4359–22,336	Ahmed et al. (2005)
		Kaempferol derivatives	321–708	Chahdoura et al. (2014a, b)
<i>O. ficus-indica/O. engelmannii</i>	Fruit	Isorhamnetin derivatives	545–2860	Salem et al. (2020)
<i>O. dillenii/O. lindheimeri/O. undulata/O. stricta</i>		Quercetin	43.20–90.50	Kuti (2004), Betancourt (2017)
		Isorhamnetin	0–50.30	Fernández-López et al. (2010)
<i>O. streptacantha</i>		Quercetin	51	Kuti (2004)
		Kaempferol	3.8	
<i>O. joconstle</i>		Protocatechuic	61..3	Osorio-Esquivel et al. (2011)
		Caffeic acid	53.2	
<i>O. humifusa</i>		Catechol	1880	Salem et al. (2020)
		Ellagic acid	1090	
<i>O. ficus-barbarica/O. robusta</i>		Ferulic acid	31.62–26.93	Kıvrak et al. (2018)
		<i>p</i> -Hydroxy benzoic acid	8.20–9.35	
<i>O. ficus indica</i>	Pulp	Piscidic acid	474.90	Gómez-Maqueo et al. (2020)
		Piscidic acid derivative	134.9	
		4-Hydroxybenzoic acid derivative	11.80	
<i>O. macrorhiza</i>		Piscidic acid	380	Chahdoura et al. (2019)
		Isorhamnetin derivatives	7	
<i>O. ficus indica/O. macrohiza/O. microdays</i>	Seeds	Feruloyl derivatives	947–356	Chahdoura et al. (2015)
<i>O. ficus indica</i>	Peels	Piscidic acid	4073	Gómez-Maqueo et al. (2020)
		Piscidic acid derivative	158.5	
		Isorhamnetin derivatives	68.5	

(continued)

**Table 17.1** (continued)

	Plant part	Main compound	Content (µg/g)	References
<i>O. macrorrhiza</i>		Quercetin derivatives	1240	Chahdoura et al. (2019)
		Isorhamnetin derivatives	1150	
		Kaempferol derivatives	100	
<i>O. engelmannii/O. microdasys</i>		Isorhamnetin derivatives	5.37–290	Chahdoura et al. (2019)
		Quercetin derivatives	0–170	
		Kaempferol derivatives	0–130	
<i>O. dillenii</i>		Kaempferol	2700	Katanić et al. (2019)
		Quercetin	600	
<i>O. albicarpa/O. ficus indica/O. hyptiacantha</i>	Cladodes	Eucomic acid	1616–13,506	Astello-García et al. (2015)
<i>O. megacantha/O. streptacantha</i>		Isorhamnetin derivatives	78.45–3310	Blando et al. (2019)
		Pscidic acid	0–1310	Ginestra et al. (2009)
<i>O. robusta/O. rastrera/O. streptacantha</i>		Isorhamnetin	99.58–326.90	Santos-Zea et al. (2011)
<i>O. undulata</i>		Kaempferol	12.90–45.6	
<i>O. lindheimeri</i>		Kaempferol	1.8	Santos-Zea et al. (2011)
<i>O. macrorrhiza</i>		Piscidic acid	3400	Chahdoura et al. (2014a, b)
		Eucomic acid	1688	
		Hexosyl ferulic acid	332	
<i>O. microdasys</i>		Isorhamnetin derivatives	2507	Chahdoura et al. (2014a, b)
		Hexosyl caffeic acid	1030	
		Hexosyl ferulic acid	852	

neuroprotective action against the oxidative injuries induced in cortical cell cultures. Rhee et al. (2008) reported that dihydroquercetin (taxifolin) isolated from *O. ficus indica* fruits displayed potent antioxidant activities in XO and DPPH assays. This compound was also effective against oxidative and microbial agents (Lee & Lee, 2010). Additionally, it exhibited significant protection on hepatocytes against alcoholic oxidative stress (Kim et al., 2017).

Protocatechuic (61.3 µg/g) and caffeic (53.2 µg/g) acids were the main compounds of *O. joconostle* fruits (Osorio-Esquivel et al., 2011a, b). Protocatechuic acid has a similar structure to gallic acid, caffeic acid, vanillic acid, and syringic

acid, which are well-known antioxidant compounds having various pharmacological activities such as antibacterial, anticancer, antiulcer, antidiabetic, antiageing, antifibrotic, antiviral, antiinflammatory, analgesic, antiatherosclerotic, cardiac, hepatoprotective, neurological and nephroprotective activities (Kakkar & Bais, 2014).

In the whole fruits of *O. ficus-barbarica*, and *O. robusta*, ferulic acid was the predominant compound with 31.6–26.9  $\mu\text{g/g}$  (Kıvrak et al., 2018). The phenolic profile of fruit seeds was determined in *O. ficus indica* (Chougui et al., 2013), *O. macrorhiza*, and *O. microdasys* (Chahdoura et al., 2015) having high amounts of feruloyl derivatives (356–947  $\mu\text{g/g}$ ). Ferulic acid and its derivatives have been widely studied for their beneficial health properties against inflammations, diabetes, cancer, cardiovascular disease, and Alzheimer's disease Karamać et al. (2007). Karamać et al. (2005) showed that ferulic acid had a better antioxidant capacity than its derivatives. The powerful antioxidant capability of ferulic acid, strongly associated with its phenolic hydroxyl group, promptly stops radical chain reactions *via* a radical scavenging mechanism (Chandrascara and Shahidi, 2010). Besides, the beneficial effects of ferulic acid were explained by this molecule's ability to interact with different cellular mechanisms that may be simultaneously and synergistically involved in its biological effect, including the regulation of signaling pathways (Chandrascara and Shahidi, 2010; Batista, 2014).

Eucomic acid was the predominant component (1616–13,506  $\mu\text{g/g}$ ) in cladodes of *O. albicarpa*, *O. ficus indica*, *O. hyptiacantha*, *O. megacantha*, and *O. streptacantha* (Ginestra et al., 2009; Astello-García et al., 2015; Blando et al., 2019). Eucomic acid was isolated from *Vanda teres* stems by Simmler et al. (2011). It was founded to increase cytochrome c oxidase activity, or expression in a human immortalized keratinocyte cell line (HaCaT), suggesting their potential as potent ingredients for anti-aging development formulations.

Piscidic acid was the predominant component in *O. macrorhiza* (3400  $\mu\text{g/g}$ ) cladodes (Chahdoura et al., 2014a, b) and in *O. ficus indica* (4073  $\mu\text{g/g}$ ) fruit peels (Gómez-Maqueo et al., 2020). In *O. ficus-indica* and *O. macrorhiza*, the fruit pulps were rich in piscidic acid ranging from 380 to 474.9  $\mu\text{g/g}$  (Gómez-Maqueo et al., 2020). Piscidic acid belongs to the family of phenylpyruvic acid derivatives, and it is marked as a potent chelator of iron with antioxidant properties (Takahira et al., 1998).

A high amount of kaempferol was detected in the fruit peels (2700  $\mu\text{g/g}$ ) of *O. dillenii* (Katanića et al., 2019) and the cladodes (1.8  $\mu\text{g/g}$ ) of *O. lindheimeri* (Santos-Zea et al., 2011). Regarding kaempferol, it was isolated from *O. ficus indica*, and it showed a potent antidepressant activity by increasing  $\beta$ -endorphin, which is an essential physiological regulator in response to depression (Park et al., 2010). Astragalin (kaempferol-3-*O*-glucoside) was also isolated from the fruit peels of *O. ficus indica*, which was tested for its anti-pneumonia activity (Elkady et al., 2020).

For *O. humifusa*, the fruits were rich in catechol (1880  $\mu\text{g/g}$ ), as reported by Salem et al. (2020). Polyphenols having catechol (1,2-dihydroxybenzene) nuclei are strong *in vitro* antioxidants due to their ability to rapidly reduce ROS and bind transition metal ions as inert complexes and regenerate the potent chain-breaking  $\alpha$ -tocopherol (Dangles, 2012).

Opuntiol (6-hydroxymethyl-4-methoxy-2H-pyran-2-one) is one of the novel active compounds isolated from *Opuntia* spp. belonging to the group of flavonoids. This compound, isolated from *O. dillenii* cladodes, had a potent antiinflammatory activity emerging as a dual inhibitor of cyclooxygenase and lipoxygenase pathways. It also suppressed ROS and cytokine levels (Siddiqui et al., 2016). Recently, opuntiol was also isolated from *O. ficus indica* cladodes by Veeramani Kandan et al. (2019a). It exhibited antiproliferative property *via* mitochondrial-dependent cell death by generating ROS in KB oral carcinoma cells. In another study, Veeramani Kandan et al. (2019b) noted that opuntiol significantly neutralized OH, O<sup>2</sup>, H<sub>2</sub>O<sub>2</sub>, and DPPH<sup>•</sup> radicals in a concentration-dependent manner. They also found that opuntiol prevented UVA-radiation-mediated oxidative stress-related biochemical changes in the mouse embryonic fibroblast cell lines (NIH-3T3).

## 2.2 Vitamins in *Opuntia* spp. and Their Antioxidant Activities

Vitamins are organic molecules required for body function and indispensable to our life. Among the 13 vitamins reported in the literature, 10 of them are found in *Opuntia* spp., including vitamin A, vitamin K1, vitamin C, vitamin B1, vitamin B2, vitamin B3,  $\alpha$ -tocopherol,  $\beta$ -tocopherol,  $\gamma$ -tocopherol, and  $\delta$ -tocopherol.

From Table 17.2, the fruit peel of *O. ficus-indica* is rich in vitamin E, having an amount of 21,820  $\mu\text{g/g}$  (Ramadan & Mörsel, 2003). The fruit pulp had a very low vitamin E content, varying from 1.11 to 1.15  $\mu\text{g/g}$  (Feugang et al., 2006). However, the oil extracted from the fruit seeds approximately had 599–1060  $\mu\text{g/g}$  of vitamin E (Ramadan & Mörsel, 2003; El Adib et al., 2015). Vitamin E was weakly detected in the fruit seeds of *O. joconostle* (32.3  $\mu\text{g/g}$ ), *O. macrorhiza* (51  $\mu\text{g/g}$ ), *O. microdasys* (69  $\mu\text{g/g}$ ), and *O. matudae* (67.1  $\mu\text{g/g}$ ). Only the flower of *O. microdasys* was investigated for its vitamin profile having 46  $\mu\text{g/g}$  of vitamin E (Chahdoura et al., 2014a, b). Vitamin E, a fat-soluble vitamin, is a group of compounds, including both tocopherols and tocotrienols. Vitamin E is a major vitamin found in *Opuntia* spp. Vitamin E can protect cell membranes against lipid peroxidation damage through different ways.

In the fruit, the highest amount of vitamin C was detected in *O. streptacantha* having 815  $\mu\text{g/g}$  (Kuti, 2004). The fruit of *O. ficus indica* contained 185–450  $\mu\text{g/g}$  of vitamin C (Ramadan & Mörsel, 2003; Kuti, 2004). This content was higher than that found in *O. stricta* (233–437  $\mu\text{g/g}$ ), *O. cochineria* (250  $\mu\text{g/g}$ ), *O. robusta* (235  $\mu\text{g/g}$ ), *O. albicarpa* (53–178  $\mu\text{g/g}$ ), *O. undulata* (145  $\mu\text{g/g}$ ), *O. megacantha* (93–137) and *O. lindheimeri* (121  $\mu\text{g/g}$ ) as reported by Fernández-López et al. (2010) and Figueroa-Cares et al. (2010). It was interesting to mention that vitamin C was absent in the fruit seeds of all *Opuntia* spp. except for *O. robusta* (361.2  $\mu\text{g/g}$ ) and *O. boldinghii* (41.5  $\mu\text{g/g}$ ) seeds (García Pantaleón et al., 2009). Vitamin C, L-ascorbic acid, or simply ascorbate is a water solubility substance. Vitamin C is a natural, free radical scavenger that can effectively scavenge various ROS species,

**Table 17.2** Distribution and contents of vitamins ( $\mu\text{g/g}$ ) in the different parts of *Opuntia* spp.

	Plant part	Vit A	Vit K1	Vit C	Vit B1	Vit B2	Vit B3	Vit	$\alpha$ -Toco	$\beta$ -Toco	$\gamma$ -Toco	$\delta$ -Toco	Total Toco (Vit E)	References
<i>O. ficus indica</i>	Cladode	NA	ND	70–220	14	60	46	ND	ND	ND	ND	ND	ND	Feungang et al. (2006)
	Peel	NA	1090	ND	ND	ND	ND	1760	2220	1740	260	260	21,820	Ramadan and Mörstel (2003)
	Pulp	NA	530	120–810	ND	ND	ND	0.67–0.69	–	0.29–0.32	0.14–0.16	0.14–0.16	1.11–1.15	Feungang et al. (2006), Tesoriere et al. (2005)
	Seeds	NA	525	ND	ND	ND	ND	56	12	330	5	5	403–1060	Ramadan and Mörstel (2003), El Adib et al. (2015)
<i>O. mattiadae</i>	Fruit	NA	NA	185–458	NA	NA	NA	NA	NA	NA	NA	NA	NA	Fernández-López et al. (2010)
	Peel	NA	NA	ND	NA	NA	NA	NA	201.40	ND	7.80	5.20	214.4	Morales et al. (2015)
	Peel + seed	NA	NA	ND	NA	NA	NA	NA	27.40	0.90	4.60	1.30	34.20	Morales et al. (2015)
	Pulp	NA	NA	316.70	NA	NA	NA	NA	1.00	ND	0.30	0.10	1.40	Morales et al. (2012)
<i>O. robusta</i>	Seeds	NA	NA	ND	NA	NA	NA	NA	1.90	0.20	64.30	0.80	67.10	Morales et al. (2012)
	Cladode	NA	NA	240.40	NA	NA	NA	NA	NA	NA	NA	NA	NA	de Wit et al. (2019)
	Peel	NA	NA	611.60	NA	NA	NA	NA	NA	NA	NA	NA	NA	de Wit et al. (2019)
	Seeds	NA	NA	361.20	NA	NA	NA	NA	NA	NA	NA	NA	NA	de Wit et al. (2019)
<i>O. jocosostle</i>	Fruit	NA	NA	235	NA	NA	NA	NA	NA	NA	NA	NA	NA	Figuroa-Cares et al. (2010)
	Pulp	NA	NA	206.30	NA	NA	NA	NA	1.60	0.10	0.50	ND	2.20	Morales et al. (2012)
	Seeds	NA	NA	–	NA	NA	NA	NA	0.90	0.10	30.70	0.60	32.30	Morales et al. (2012)
<i>O. stricta</i>	Cladode	7112	NA	29	NA	NA	NA	NA	NA	NA	NA	NA	2314	Izuegbuna et al. (2019)
	Fruit	NA	NA	233–437	NA	NA	NA	NA	NA	NA	NA	NA	NA	Fernández-López et al. (2010), Kuti (2004)
<i>O. boldinghii</i>	Cladode	NA	NA	121.10	NA	NA	NA	NA	NA	NA	NA	NA	NA	Moreno Álvarez et al. (2009)
	Seeds	NA	NA	41.50	NA	NA	NA	NA	NA	NA	NA	NA	NA	García Pantaleón et al. (2009)
<i>O. lindheimeri</i>	Fruit	NA	NA	121	NA	NA	NA	NA	NA	NA	NA	NA	NA	Kuti (2004)

(continued)

Table 17.2 (continued)

	Plant part	Vit A	Vit K1	Vit C	Vit B1	Vit B2	Vit B3	Vit	$\alpha$ -Toco	$\beta$ -Toco	$\gamma$ -Toco	$\delta$ -Toco	Total Toco (Vit E)	References
	Pulp	NA	NA	680.30	NA	NA	NA	NA	NA	NA	NA	NA	NA	Monroy-Gutiérrez et al. (2017)
<i>O. albicarpa</i>	Fruit	NA	NA	53–178	NA	NA	NA	NA	NA	NA	NA	NA	NA	Figuroa-Cares et al. (2010)
	Pulp	NA	NA	238.30	NA	NA	NA	NA	NA	NA	NA	NA	NA	Monroy-Gutiérrez et al. (2017)
<i>O. streptacantha</i>	Fruit	NA	NA	815	NA	NA	NA	NA	NA	NA	NA	NA	NA	Kuti (2004)
	Pulp	NA	NA	109	NA	NA	NA	NA	NA	NA	NA	NA	NA	Monroy-Gutiérrez et al. (2017)
<i>O. microdasys</i>	Flower	NA	NA	NA	NA	NA	NA	NA	36	2.80	3.6	3	46	Chahdoura et al. (2014a, b)
	Seeds	NA	NA	NA	NA	NA	NA	NA	53	13.6	2.4	0.6	69	Chahdoura et al. (2019)
<i>O. cochimera</i>	Fruit	NA	NA	250	NA	NA	NA	NA	NA	NA	NA	NA	NA	Figuroa-Cares et al. (2010)
<i>O. megacantha</i>	Fruit	NA	NA	93–137	NA	NA	NA	NA	NA	NA	NA	NA	NA	Figuroa-Cares et al. (2010)
<i>O. undulata</i>	Fruit	NA	NA	145	NA	NA	NA	NA	NA	NA	NA	NA	NA	Fernández-López et al. (2010)
<i>O. engelmannii</i>	Pulp	NA	NA	NA	NA	NA	NA	NA	0.15	ND	0.27	NA	0.43	Melgar et al. (2017)
<i>O. dillenii</i>	Pulp	NA	NA	360	6	7	ND	NA	NA	NA	NA	NA	0.02	Abdallah (2018)
<i>O. eliator</i>	Pulp	NA	NA	150.90	NA	NA	NA	NA	NA	NA	NA	NA	NA	Moreno Álvarez et al. (2008)
<i>O. chavena</i>	Pulp	NA	NA	396.40	NA	NA	NA	NA	NA	NA	NA	NA	NA	Monroy-Gutiérrez et al. (2017)
<i>O. elata</i>	Pulp + peel	NA	NA	897	NA	NA	NA	NA	NA	NA	NA	NA	NA	Reis et al. (2017)
<i>O. macrorhiza</i>	Seeds	NA	NA	NA	NA	NA	NA	NA	49	0.3	2.1	NA	51	Chahdoura et al. (2019)

ND not detected, NA not available, *Vit* vitamin, *Toco* tocopherol

give off semi dehydroascorbic acid, clearing  $^1\text{O}_2$ , and reduce sulfur radicals (Amitava & Kimberly, 2014).

Vitamin K1 was present in all fruit parts of *O. ficus-indica*, ranging from 525 to 1090  $\mu\text{g/g}$  (Ramadan & Mörssel, 2003; Kuti, 2004; Feugang et al., 2006; El Adib et al., 2015). Vitamin K is known as blood clotting factors because the name derived from the German word “koagulation” means blood clotting. Three different types of vitamin K are known, namely; K1 (phylloquinone), K2 (menaquinone), and K3 (menadione). K1 form is most prevalent and needed for photosynthesis in plants (Olorunnisola Olubukola et al., 2019). Vitamin K is essential for blood clotting activity (Shearer and Newman 2017).

Vitamins B were weakly present in the cladode of *O. ficus-indica* having B1 = 14  $\mu\text{g/g}$ ; B2 = 60  $\mu\text{g/g}$  and B3 = 46  $\mu\text{g/g}$  (Feugang et al., 2006) as well as in the fruit pulp of *O. dillenii* with B1 = 6  $\mu\text{g/g}$  and B2 = 7  $\mu\text{g/g}$  (Abdallah, 2018). Vitamins B is a class of eight water-soluble vitamins (B1, B2, B3, B5, B6, B9, and B12) that play essential roles in cell metabolism (Olorunnisola Olubukola et al., 2019). Vitamin B1 belongs to the group of vitamin B complex and is known as thiamin. Vitamin B1 plays an essential role in human health. It is involved in energy production from carbohydrates and fats (Kala, 2003). Vitamin B1 acts as a co-enzyme precursor of some key enzymes of carbohydrate metabolism. It also helps in the structural development of brain cells, and it is involved in the detoxification of alcohol (Ba, 2008). Vitamin B2 is also known as riboflavin. It is involved in energy metabolism. Vitamin B2 recycles glutathione, which is the most crucial antioxidant which protects against free radicals in the body. It also promotes iron metabolism, and its deficiency increases the risk of anemia as iron is an essential element for red blood cell production (Said & Ross, 2014). Vitamin B3 is also known as Niacin. Niacin is a group of compounds having vitamin activity. Vitamin B3 comprises nicotinic acid, nicotinamide, and numerous enzymatic forms (Long et al., 2015). NAD and NADP are two distinct forms of vitamin B3, and they are primarily involved in the production of energy from dietary proteins, carbohydrates, and fats (Lesková et al., 2006). NAD, NADP, and niacin-containing enzymes are scavengers of free radicals and protect tissues from oxidative damage (Lanska, 2009).

Vitamin A (7112  $\mu\text{g/g}$ ) was only detected in *O. stricta* cladodes (Izuegbuna et al., 2019). Vitamin A is a class of fat-soluble organic compounds, which includes retinol, retinal, retinoic acid, and several provitamin A ( $\beta$ -carotene). Vitamin A can react with free radicals (especially  $\text{O}_2$ ) and peroxy radicals to show its antioxidant property (Amitava & Kimberly, 2014).

### 2.3 Sterols in *Opuntia* spp. and Their Antioxidant Activities

The biological properties of phytosterols, especially their capacity for reducing blood cholesterol levels, are the main reason behind their use as food ingredients. From Table 17.3, Ramadan and Mörssel (2003) reported that  $\beta$ -sitosterol as the major sterol extracted from different parts of the fruit oils: pulp, skin, and seeds, with



**Table 17.3** Distribution and contents of sterols ( $\mu\text{g/g}$ ) in the different parts of *Opuntia* spp.

Plant part	Campesterol	Stigmasterol	Lanosterol	$\beta$ -Sitosterol	$\Delta^5$ -Avenasterol	$\Delta^7$ -Avenasterol	Ergosterol	Cholesterol	References
<i>O. ficus indica</i>	Seeds	1660	300	280	6750	290	ND	ND	Ramadan and Mörsel (2003)
	Pulp	8740	730	760	1120	1430	ND	ND	Ramadan and Mörsel (2003)
	Peel	8760	2120	1660	21,100	2710	680	ND	Ramadan and Mörsel (2003)

ND not detected

content ranging from 6750 to 21,100  $\mu\text{g/g}$ . Campesterol was present in the pulp, seed, and skin, in an amount of 1660–8760  $\mu\text{g/g}$ . Other sterols were found in small quantities, notably stigmasterol, lanosterol,  $\Delta 5$ -avenasterol,  $\Delta 7$ -avenasterol, ergosterol. Jiang et al. (2006) had isolated opuntisterol,  $\beta$ -sitosterol, daucosterol, and 7-oxositosterol from *O. dillenii* stems.  $\beta$ -Sitosterol isolated from *Polygonum hydro-piper* possessed strong anticholinesterase and antioxidant potentials. The anticholinesterase effect of  $\beta$ -sitosterol produced inhibition by strongly binding to the active sites of AChE and BChE. In addition to cholinesterase inhibitions, free radicals scavenging potentials of  $\beta$ -sitosterol may be useful in managing several neurological disorders (Ayaz et al., 2017). Stigmasterol present in the bark of *Butea monosperma* showed a decrease in hepatic lipid peroxidation. It increased catalase activities, superoxide dismutase, and glutathione, thereby suggesting its antioxidant property (Panda et al., 2009). Yoshida and Niki (2003) found that  $\beta$ -sitosterol, stigmasterol, and campesterol exerted antioxidant effects on the oxidation of methyl linoleate in solution and its effect decreased in the order of campesterol approximately =  $\beta$ -sitosterol > stigmasterol. Phytosterol also suppressed the oxidation and consumption of  $\alpha$ -tocopherol in  $\beta$ -linoleoyl-gamma-palmitoyl phosphatidylcholine liposomal membranes. These authors concluded that phytosterol chemically acts as an antioxidant, a modest radical scavenger, and physically as a stabilizer in the membranes. In another study, Rajavel et al. (2017) reported that  $\beta$ -sitosterol and daucosterol alone could be considered safe and potential drug candidates for lung cancer treatment by perturbing cell cycle and inducing apoptotic cell death. The intravitreal injection of lanosterol nanoparticles saved lens structure collapse at an early stage in Shumiya cataract rats (Nagai et al., 2020). Ergosterol isolated of *Hygrophoropsis aurantiaca* was tested *in vitro* for its antiproliferative activity (Nowak et al., 2016).

## 2.4 Carotenoids in *Opuntia* spp. and Their Antioxidant Activities

Carotenoids are a type of tetraterpenoids. They have been reported to show antioxidant activities through quenching  $^1\text{O}_2$  and eliminating harmful free radicals (Di Mascio et al., 1989). They may also protect immune cell membrane lipids from oxidative damage, thus ensuring communication signals between cells and receptors on the cell membrane to maintain normal cell function and enhance human immunity (Zhou et al., 2012). In *O. ficus indica* fruit, 13 carotenoids were identified (all-E-violaxanthin, all-E-neoxanthin, 9Z-violaxanthin, all-E-antheraxanthin, 9Z-neoxanthin, all-E-lutein, all-E-zeaxanthin, lutein-5,6-epoxide, all-E- $\beta$ -cryptoxanthin, all-E- $\alpha$ -carotene, all-E- $\beta$ -carotene, 9Z- $\beta$ -carotene, and lycopene). The main carotenoid in *O. ficus indica* fruit was (all-E)-lutein with 7.67–11.3  $\mu\text{g/g}$  in the pulp, 3.16–3.32  $\mu\text{g/g}$  in fruit, and 2.01–2.04  $\mu\text{g/g}$  in peel (Cano et al., 2017). In cladodes, 3 carotenoids were identified, namely lutein (175–270  $\mu\text{g/g}$ ),  $\beta$ -carotene

(142–207  $\mu\text{g/g}$ ), and  $\alpha$ -cryptoxanthin (32–77  $\mu\text{g/g}$ ) (González-Cruz et al., 2012). So far, the carotenoid composition of the other *Opuntia* spp. remains to be determined.

The protective role of lutein against lipid peroxidation was investigated in membranes made of raft-forming mixtures and in models of photoreceptor outer segment membranes and compared with their antioxidant activity in homogeneous membranes composed of unsaturated lipids (Wisniewska-Becker et al., 2012). Zeaxanthin has been less studied than its isomer lutein. However, zeaxanthin has been shown to have several beneficial effects for human health due to its ability to quench free radicals, exert antioxidant effects, and decrease inflammation (Murillo et al., 2019). Violaxanthin, a xanthophyll carotenoid present in plants' photosynthetic apparatus, is rapidly and reversibly de-epoxidized into zeaxanthin *via* the intermediate antheraxanthin under high-light stress (Horton et al., 2005).  $\beta$ -Carotene has potential antioxidant biological properties due to its chemical structure and interaction with biological membranes (Riccioni, 2009). It is well-known that  $\beta$ -carotene quenches singlet oxygen with a multiple higher efficiency than  $\alpha$ -tocopherol (Di Mascio et al., 1989).  $\beta$ -Cryptoxanthin is an antioxidant *in vitro* and appears to be associated with decreased risk of some cancers and degenerative diseases. Besides, many *in vitro* animal models, and human studies suggest that  $\beta$ -cryptoxanthin-rich foods may have an anabolic effect on bone and may help delay osteoporosis (Burri et al., 2016). Lycopene exhibits a high physical quenching rate of  $^1\text{O}_2$  (Di Mascio et al., 1989), directly related to its antioxidant activity. The rates for other lipophilic antioxidants, namely  $\beta$ -carotene and  $\alpha$ -tocopherol, are about 2-fold and 100-fold lower. The quenching efficacy of lycopene is related to the opening of the  $\beta$ -ionone ring to an open-chain form. Lycopene can also play a role in scavenging HOCl (Pennathur et al., 2010). This acid contributes to the pathology of atherosclerosis, inflammatory disease, respiratory stress, acute vasculitis, and cancer. The oxidation of lycopene *via* HOCl is accompanied by a change in its colour from red to colourless (Amarowicz, 2011).

## 2.5 Betalains in *Opuntia* spp. and Their Antioxidant Activities

Betalains from *Opuntia* fruits exhibited strong antioxidant activities in the biological environment by inhibiting lipid peroxidation and heme decomposition at very low concentrations (Gengatharan et al., 2015). Betalains also counteract lipoperoxidases, damaging gastrointestinal cells during food digestion (Cai et al., 2003). Betalains are excellent radical scavengers with an antioxidant activity three to four times higher than ascorbic acid, rutin, and catechin (Cai et al., 2005), twice higher than that measured for pear, apple, tomato, banana, and white grape, and from the same order as pink grapefruit, red grape, and orange (Strack et al., 2003).

From Table 17.4, the fruits, pulps, and peels of *O. ficus-indica* contained different betalains. The betacyanins identified in *O. ficus-indica* fruits, pulps and peels included betanin (21.4–1812  $\mu\text{g/g}$ ), isobetainin (tr.-153.8  $\mu\text{g/g}$ ), betanidin

**Table 17.4** Distribution and contents of carotenoids in the different parts of *Opuntia* spp.

Carotenoids ( $\mu\text{g/g}$ )	<i>O. ficus-indica</i>			
	Fruit <sup>a</sup>	Pulp <sup>a</sup>	Peel <sup>a</sup>	Cladode <sup>b</sup>
all-E-Violaxanthin	0.22–0.23	0.05–0.32	0.87–0.93	NA
all-E-Neoxanthin	0.07–0.12	0.02–0.12	0.34–0.59	–
9Z-Violaxanthin	0.10–0.08	0–0.12	0.29	–
all-E-Antheraxanthin	0.10–0.11	0.05–0.07	0.39–0.40	–
9Z-Neoxanthin	Tr	Tr	Tr	–
all-E-Lutein	3.16–3.32	2.01–2.04	7.67–11.32	175–270
all-E-Zeaxanthin	0.14–0.13	0.12–0.14	0.53–0.63	–
Lutein-5,6-epoxide	0.02–0.13	0.04	0.11–0.63	–
all-E- $\beta$ -Cryptoxanthin	ND	ND	0.05–0.08	–
$\alpha$ -Cryptoxanthin	NA	NA	NA	32–77
all-E- $\alpha$ -Carotene	0.01–0.03	0–0.05	0.08–0.10	–
all-E- $\beta$ -Carotene	0.53–0.65	0.37–0.79	1.73–2.00	142–207
9Z- $\beta$ -Carotene	0.08–0.06	0–0.06	0.21–0.26	–
Lycopene	Tr	Tr	0–0.45	–

<sup>a</sup>Data from Cano et al. (2017)

<sup>b</sup>González-Cruz et al. (2012)

(tr.-37.4  $\mu\text{g/g}$ ), gomphrenin I (tr.-132.7  $\mu\text{g/g}$ ), and neobetanin (0–61.9  $\mu\text{g/g}$ ) as reported by García-Cayueta et al. (2019). These authors also noted the presence of conjugates of betalamic acid with several amino acids is reported in fruits, pulps, and peels, corresponding to portulacaxanthin I (tyrosine, 8.30–19.6  $\mu\text{g/g}$ ), portulacaxanthin III (lysine, 4.2–84.1  $\mu\text{g/g}$ ), vulgaxanthin III (asparagin, tr.-28.9  $\mu\text{g/g}$ ), vulgaxanthin I (glutamine, 10.20–57.30  $\mu\text{g/g}$ ), vulgaxanthin II (glutamic acid, 8.4–16.3  $\mu\text{g/g}$ ), Bx-amino butyric acid (7.5–17.9  $\mu\text{g/g}$ ), indicaxanthin (proline, 19–254  $\mu\text{g/g}$ ), Bx-tryptophan (8–17.9  $\mu\text{g/g}$ ). Using *O. ficus-indica* fruits as a betalain source is of great interest because they are highly flavored, with better nutritional properties than red beetroot. So far, the betalain composition of the other *Opuntia* spp. remains to be determined.

Generally, betalains can be classified into betacyanins (red-violet) or betaxanthins (yellow-orange). Betacyanins are derivatives of betanidin, an iminium adducts of betalamic acid, and cyclo-DOPA, whereas betaxanthins result from the condensation of  $\alpha$ -amino acids or amines with betalamic acid (Delgado-Vargas et al., 2000). The active cyclic amine group of betalains functions as a hydrogen donor and confers reducing properties to these compounds (Kanner et al., 2001). The betacyanins such as betanin and betanidin had enhanced antioxidant capacity compared to betaxanthins due to the presence of a phenolic ring, which increases their electron transfer capability (Stintzing et al., 2005). Sreekanth et al. (2007) showed that betanin purified from *O. ficus-indica* fruits had induced apoptosis in human chronic myeloid leukemia cell line-K562 (Table 17.5).

**Table 17.5** Distribution and contents of betalins in the different parts of *Opuntia* spp.

Betalins ( $\mu\text{g/g}$ )	<i>O. ficus-indica</i>		
	Whole fruit <sup>a</sup>	Pulp <sup>a</sup>	Peel <sup>a</sup>
<i>Betaxanthin</i>			
Portulacaxanthin I	8.80–19.60	9–17	8.30–14.60
Portulacaxanthin III	84.10–11.20	72.70–4.70	4.20–48.30
Bx-unknown	0–16.70	0–14.30	2.50–7.50
Vulgaxanthin III	Tr.-28.90	Tr.-25	Tr.-15
Vulgaxanthin I	16.40–57.30	16.80–38.70	10.20–41
Vulgaxanthin II	8.40–16.30	9.40–12.80	8.60–16.20
Bx-amino butyric acid	7.5–17.9	9.2–14.3	7.5–12.2
Indicaxanthin	46.6–254.1	207–221.8	19–87.2
Bx-tryptophan	8.4–17.9	9–13.4	8–17.2
<i>Betacyanin</i>			
Betanin	21.4–1812.9	26.2–1171.3	27.8–880.9
Isobetanin	Tr.-153.8	Tr.-105.8	Tr.-47.5
Betanidin	Tr.-37.4	Tr.-37.3	Tr.-26.3
Gomphrenin I	Tr.-132.7	Tr.-118.9	Tr.-51.6
Neobetanin	0–57.3	0–61.9	0–19
Total betaxanthin	134.8–487.9	206.8–405.1	69.1–252.3
Total betacyanin	21.4–2176	26.2–2066.9	27.8–1021.7
Total betalain	156.2–2422.5	240.5–2273.6	125.7–1197.4

<sup>a</sup> García-Cayuela et al. (2019)

Additionally, betanin was also isolated from *O. elatior* fruits by Sutariya and Saraf (2017). They indicated that this compound could effectively attenuate renal fibrosis in diabetic rats by regulating oxidative stress and TGF- $\beta$  pathways. Recently, González-Ponce et al. (2020) found that betacyanins of *O. robusta* and *O. streptacantha* fruits were found to protect against acetaminophen-induced acute liver failure. On the other hand, the betaxanthins, such as indicaxanthin, have been the experimental sound work object over the latest years. Like many phytochemicals, indicaxanthin is a redox-active compound and has been shown to act as an antioxidant in some *in vitro* studies (Turco Liveri et al., 2009). Interestingly, thanks to its charged portions, ionizable groups, and lipophilic moieties, it is amphiphilic at physiological pH and has been demonstrated to interact with cell membranes (Turco Liveri et al., 2009). Allegra et al. (2018) mentioned that the isolated indicaxanthin from *O. ficus indica* fruits inhibited human melanoma cell proliferation *in vitro* and markedly impair tumor progression *in vivo*.

## 2.6 Other Constituents

Other bioactive compounds were isolated from *Opuntia* spp. having several biological effects (Table 17.6). Fernández et al. (1992) isolated pectin from *Opuntia* spp. which decreased blood lipid level and peroxidative status. It is chemically a polysaccharide, consisting of a linear chain of linked galacturonic acid. Pectin is widely used as a texturizer and stabilizer in a variety of foods and other industries. Oh and Lim (2006) isolated a glycoprotein from *O. ficus-indica* fruit, which decreased plasma lipid level through scavenging of intracellular radicals in triton WR-1339-induced mice. An opuntioside, isolated from *O. dillenii* cladode, had a potent anti-inflammatory activity (Siddiqui et al., 2016).

Kim et al. (2017) isolated lignans from *O. ficus-indica* seeds, which protected rat primary hepatocytes and HepG2 cells against ethanol-induced oxidative stress. Cruz Filho et al. (2019) isolated lignins from *O. ficus-indica* and *O. cochenillifera* cladodes, which induced the activation and proliferation of splenocytes and the production of cytokines in mice. The authors also found that *O. cochenillifera* lignin presented more phenolic amount and antioxidant activities (using DPPH<sup>•</sup>, ABTS, NO assays, and total antioxidant activity) than *O. ficus-indica*. Both lignins showed high cell viability (>96%) and cell proliferation. Lignins induced high TNF- $\alpha$ , IL-6, and IL-10 production and reduced NO release.

In a recent study, Surup et al. (2021) reported that the novel opuntisine A, a cyclopeptide alkaloid isolated from *O. stricta* fruits, showed moderate activity against the Gram-negative bacterium *Escherichia coli*, but no further antibacterial, antifungal, nor cytotoxic effects were reported.

## 3 Methods for the Antioxidant Activity Evaluation of *Opuntia* spp.

A wide variety of *in vitro* and *in vivo* methods was used for antioxidant activity evaluation of *Opuntia* spp. *in vitro* assays are mainly concerned with the free radical or oxide scavenging capacity of the antioxidants. In the current literature, the major *in vitro* methods used for plant samples are DPPH<sup>•</sup>, ABTS, ORAC, TRAP, TEAC, FRAP, CUPRAC, and PCL (Alam et al., 2013). The principal advantage of these methods is speed and simplicity. However, the disadvantage is that their results are influenced by many factors, such as antioxidants and interactions, interference materials, pH, action time, producing systems for free radicals, etc. For *Opuntia* spp., the DPPH<sup>•</sup>, FRAP, ABTS, and ORAC methods are frequently used to evaluate antioxidant activity (Valente et al., 2010; Bensadón et al., 2010; Alimi et al., 2011; Ammar et al., 2012; Dib et al., 2013; Benayad et al., 2014; Sánchez Gullón et al., 2014; Astello-García et al., 2015; Haile et al., 2016; Cano et al., 2017; Boutakiout et al., 2018; Blando et al., 2019; Izuegbuna et al., 2019; Missaoui et al., 2020; Aruwa et al., 2019). The antioxidant properties of cactus pear were reported due to

**Table 17.6** Characteristics of bioactive compounds isolated from *Opuntia* spp.

Active compound/chemical class	Source	Assay	Activity	Results/mechanism of action	References
Isorhamnetin (Flavonoid)	<i>O. ficus-indica</i>	<i>In vivo</i> NOS	Anti-inflammatory activity	Inhibition of NO production in RAW264.7 cells	Antunes-Ricardo et al. (2015)
Isorhamnetin derivatives (Flavonoid) RI = glucosyl-rhamnosyl-rhamnoside. RI = glucosyl-rhamnosyl-pentoside. RI = glucosyl-pentoside. RI = glucosyl-rhamnoside					
Quercetin (Flavonoid)	<i>O. ficus-indica</i>	<i>In vitro</i> DPPH, XO and LPO	Antioxidant activity Neuroprotective effect	Quercetin, quercetin 3-methyl ether and dihydroquercetin markedly inhibited lipid peroxidation and scavenged DPPH. Quercetin 3-methyl ether appeared to have the most potent neuroprotective action	DoK-Go et al. (2003)
Quercetin-3-methyl ether (Flavonoid)					
Dehydroquercetin = Taxifolin (Flavonoid)					
Quercitrin (Flavonoid)	<i>O. ficus-indica</i>	<i>In vivo</i> TST	Antidepressant activity	Potent antidepressant effect	Park et al. (2010)
Kaempferol (Flavonoid)					
Kaempferol-3-O-glucoside = Astragalin (Flavonoid)	<i>O. ficus-indica</i>	<i>In vitro</i> MIC	Anti-pneumonia activity	Potent antibacterial effect against some pneumonia pathogens	Elkady et al. (2020)
Opuntiol = 6-hydroxymethyl-4-methoxy-2H-pyran-2-one (Flavonol)	<i>O. dillenii</i>	<i>In vivo</i> COX and SOX	Anti-inflammatory activity	Inhibitor of COX and LOX pathways. It also suppressed ROS and cytokine levels	Siddiqui et al. (2016)
Opuntioside					

Pectin (Polysaccharide)	<i>O. ficus indica</i>	<i>In vivo</i> LDL	Anthyperlipidemic effect	Decrease of blood lipid level and peroxidative status	Fernández et al. (1992)
Glycoprotein	<i>O. ficus indica</i>	<i>In vivo</i> LDL, HDL, TBARS, CAT, SOD, GPx and NO	Anthyperlipidemic effect	Decrease of blood lipid level and peroxidative status	Oh and Lim (2006)
Lignans	<i>O. ficus indica</i>	<i>In vivo</i> ROS	Hepatoprotective effect	Protection of rat primary hepatocytes and HepG2 cells against ethanol-induced oxidative stress	Kim et al. (2017)
Lignins	<i>O. ficus indica</i> <i>O. cochenillifera</i>	<i>In vitro</i> DPPH, ABTS, NO, CFSE, Annexin V-Fluorescein isothiocyanate and propidium iodide-PE	Antioxidant activity Cytotoxicity activity Anti-inflammatory activity	<i>O. cochenillifera</i> lignin presented more phenolic amount and antioxidant activities than <i>O. ficus-indica</i> . Both lignins showed high cell viability (>96%) and cell proliferation. Lignins induced high TNF- $\alpha$ , IL-6 and IL-10 production and reduced NO release	Cruz Filho et al. (2019)
Opuntocine A (Cyclopeptide alkaloid)	<i>O. ficus indica</i>	<i>In vitro</i> MIC	Antibacterial activity	Moderate activity against the Gram-negative bacterium <i>Escherichia coli</i> , but no further antibacterial, antifungal nor cytotoxic effects	Surup et al. (2021)

*ABTS* 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), *CAT* catalase, *CFSE* carboxyfluorescein succinimidyl ester, *COX* cyclooxygenase, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *GPx* glutathione peroxidase, *HDL* high density lipoprotein, *IL-6* interleukin-6, *IL-10* interleukin-10, *LDL* low density lipoprotein, *LPO* lipid peroxidation, *LOX* lipoxygenase, *LOX* lipoxygenase, *MIC* minimum inhibitory concentration, *NO* nitric oxide, *NOS* nitric oxide synthase, *ROS* reactive oxygen species, *SOD* superoxide dismutase, *SOX* sulfur oxide, *TBARS* Thiobarbituric acid reactive substances, *TNF- $\alpha$*  tumor necrosis factor alpha, *TST* tail suspension test, *XO* xanthine oxidase



betalains' presence (betanin and betaxanthin) (Osuna-Martínez et al., 2014). Various *in vitro* studies have reported the beneficial effect of colorless phenolics and betalains (Tesoriere et al., 2004).

For all *in vivo* methods, samples to be tested are administered to the testing animals (rats, mice, etc.) at a definite dosage regimen, usually described by the respective method. After a specified period, the testing animals are sacrificed, and tissues or blood are used for the assay (Alam et al., 2013). The *in vivo* methods reported in the existing literature for the antioxidant activity evaluation of plant samples include enzyme activity assays (Alam et al., 2013) and cellular antioxidant activity assays (Wolfe et al., 2008). Enzymes involved *in vivo* metabolism are important to maintaining cellular redox status (López-Alarcón & Denicola, 2013). In the current literature, the major enzyme activity assays used for plant samples are GSH, GSHPx, GSt, SOD, CAT, GGT, GR, LDL, and LPO (Alam et al., 2013). In this way, the *in vivo* antioxidant and inhibitory effects on LDL peroxidation were investigated using extracts from *O. dillenii* (Wolfe et al., 2008), *O. robusta* (Budinsky et al., 2001), *O. joconostle* (Osorio-Esquivel et al., 2012), and *O. ficus indica* (Linarès et al., 2007) fruits. Likewise, glycoprotein isolated from *O. ficus-indica* exerted potent antioxidant and hypolipidemic properties evidenced by a protective effect on mice (Oh & Lim, 2006). Additionally, Wolfe et al. (2008) concluded that *O. dillenii* fruit's hypolipidemic effect might result from pectin.

On the other hand, due to the ethical issues with human study and the time-consuming and expensive characters of animal experiments, these methods are widely recognized as a reasonably reliable method for the antioxidant activity evaluation of phytochemicals and extracts. Several cell models were used for evaluating the antioxidant activities of *Opuntia* spp. phytochemicals. Sreekanth et al. (2007) showed that betanin purified from *O. ficus-indica* fruits had induced apoptosis in human chronic myeloid leukemia cell line-K562. Opuntiol was also isolated from *O. ficus indica* cladodes by Veeramani Kandan et al. (2019a), and exhibited antiproliferative property *via* KB oral carcinoma cells. Serra et al. (2013) deduced that the protective effect of *O. ficus-indica* and *O. robusta* juice against human colon cancer cell line (HT29) might result from betacyanins, ferulic acid, and isorhamnetin derivatives. Naselli et al. (2014) studied *O. ficus-indica* fruit aqueous extract and its betalain pigment indicaxanthin on the proliferation of the human colon cancer cell line Caco-2.

## 4 Mechanism of the Antioxidant Activity

In general, antioxidants act in one or more of the following pathways: direct reaction with ROS/RNS as a "free radical scavenger", inhibition of oxidant enzymes, interaction with redox signaling pathways, chelate with transitional metals yielding less oxidative damage (Zou et al., 2016). *Opuntia* spp. bioactive compounds may directly react with ROS and/or RNS. ROS/RNS, including mainly  $H_2O_2$ ,  $O_2^-$ ,  $^-\text{OH}$ ,  $^1\text{O}_2$ , NO, ONOO $^-$ , HOCl,  $\text{CO}_3^{2-}$  and ROO $^-$  are involved in the growth, differentiation, progression, and death of cells and can react with proteins, enzymes,

membrane lipids, nucleic acids, and other small molecules. The bioactive compounds of *Opuntia* spp. may act as a safeguard against the accumulation of ROS/RNS and eliminate them from the system. These compounds are good scavengers of ROS/RNS by reacting with free radicals to reduce their activity and prevent further chain reactions (Zou et al., 2016).

*Opuntia* spp. bioactive compounds may chelate with transitional metals and show antioxidant activities. Lipid peroxidation is one of the major causes of oxidative cell damage. In oxidation, the action of metal ions, as  $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$  ions, is the leading cause of lipid peroxidation reactions (Dávalos et al., 2003). Butera et al. (2002) reported that the interactions between betanin in *O. ficus indica* fruits and copper ions could play some role in protecting the  $\text{Cu}^{2+}$ -mediated oxidation of LDL by betanin-containing extracts.

*Opuntia* spp. bioactive compounds may exert their antioxidant capacity by inhibiting the oxidant enzymes *via* their bioactive compounds. Oxidant enzymes, as NOS, LOX, XO, COX, NOX, and MPO, have played important roles in redox reactions of a biological system and are also the main promoters of cellular ROS/RNS (López-Alarcón & Denicola, 2013). The inhibition of XO has been suggested to be one of the key mechanisms of antioxidant action in natural products (López-Alarcón & Denicola, 2013). Rhee et al. (2008) reported that quercetin's antioxidant activity using the XO assay system is more potent than that of using the DPPH' radical scavenging system. Indicaxanthin isolated from *O. ficus indica* inhibits NOX activation issue of inflammatory mediators and prevents the increase of epithelial permeability in IL-1 $\beta$ -exposed Caco-2 cells (Tesoriere et al., 2014). Furthermore, taxifolin, isolated from *O. ficus indica* fruits, inhibited NO production, and blocked NOS expression in the LPS-stimulated RAW264.7 cells (Rhee et al., 2008).

*Opuntia* spp. bioactive compounds may activate the Nrf-2 transcription factor and inhibit NF- $\kappa$ B of the redox signaling pathway to exhibit their antioxidant activity. According to Arredondo et al. (2010), isolated polyphenols like quercetin and extracts from natural products show an antioxidant response *via* activation of Nrf-2 (Tanigawa et al., 2007). Tesoriere et al. (2013) reported that indicaxanthin isolated from *O. ficus indica* fruits inhibits NF- $\kappa$ B-dependent release of inflammatory mediators and prevents the increase of epithelial permeability in IL-1 $\beta$ -exposed Caco-2 cells. Similarly, taxifolin significantly inhibited the transcriptional activity of NF- $\kappa$ B and activator protein-1 (AP-1) in LPS-stimulated RAW264.7 cells (Rhee et al., 2008).

## 5 Conclusion and Further Suggestions

*Opuntia* spp. are an interesting and rich source of bioactive compounds; their properties indicate their potential for pharmaceutical purposes. Further studies are still needed to (1) clarify the molecular processes and signaling pathways by which the bioactive compounds of *Opuntia* spp. involving *in vivo* system, (2) determine the synergism and/or antagonism between the different bioactive compounds detected in *Opuntia* spp., and (3) discover new bioactive compounds and their antioxidant activities from *Opuntia* species.

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# Chapter 18

## Natural Antimicrobial Molecules from *Opuntia* spp. and Their Role in Poultry Nutrition



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**Abstract** Food pathogens are causing many diseases with significant effects on human health and the economy. The Centers for Disease Control and Prevention (CDC) reported that approximately 48 million Americans get sick, 128,000 are hospitalized, and 3000 die each year from food poisoning. As specified by the CDC, the known pathogens (bacteria, viruses, and parasites) account for most foodborne illnesses, hospitalization, and death in the United States. *Opuntia* spp. are, to a great extent, dispersed in Africa, Asia, America, and the Mediterranean bowl. This plant is utilized as a dietary and pharmaceutical operator in a different dietary and value-added push. Even though contrasts within the phytochemical composition exist between wild and tamed (*Opuntia ficus-indica*) *Opuntia* spp., all *Opuntia* vegetative (pear, roots, cladodes, pulp, and seeds) display useful properties as anticancer and antimicrobial agents (flavonoids, and ascorbate), and as antimicrobial (phenolic

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acids) agents. Other phytochemical components (soluble fibers and biopeptides) have been shared in the antimicrobial character of *Opuntia* spp. Bioactive peptides are composed of a few amino acids linked by covalent bonds called amide bonds or peptides. Depending on their grouping of amino corrosive, these peptides may influence the body's major frameworks such as resistant, stomach related, cardiovascular, and anxious system. The bioactive peptides can be utilized as valuable nourishment additives. Their grouping measures contrast from 2 to 20 amino corrosive buildups, but a few peptides have a long chain of amino corrosive (lunasin 43 amino corrosive). Bioactive peptides have the plausibility to be utilized as characteristic nourishment added substance and pharmaceuticals constituents to avoid nourishment harming and nourishment added items due to their antioxidant and/or antimicrobial impacts. Moreover, natural molecules from *Opuntia* spp. ought to be planned *in vitro*, *in vivo*, *in situ*, and in a way to assess the risks to humans, animals, and food chains. This chapter is an upgrade on the bioactive molecule's properties of *Opuntia* spp. and their potential intrigued as antimicrobial.

**Keywords** Pathogen · Foodborne illnesses · Bioactive molecules · Antimicrobial · Antioxidants

## 1 Introduction

Plants with valuable characteristics are recognized in conventional medicine. Supplementation of food or feed by these plants has many beneficial properties in ethnic or traditional food, yet as in animals' dietary. The plants rich in antioxidants, antimicrobials, and bioactive molecules are considered essential sources and natural factories for locating and isolating these bioactive molecules. This successively has driven to the greatest demand for natural and nutraceutical food origin. Nowadays, the search favors natural molecules to cut back costs and slight side effects. The increasing interest in medical practice encourages the use of nutraceuticals, bioactive compounds of vegetable origin with important nutritional values. Amongst the medicinal plants, *Opuntia ficus-indica* (L.) Miller is widely known for its beneficial properties. Aragona et al. (2018) reviewed the master classes of *Opuntia* ingredients and their medical benefit emphasizing their biological effects, essentially these having the utmost promising expected health benefits and therapeutic impacts on fish and mammals. Nopal (*Opuntia* spp.) is by excellence the foremost utilized cactus in human and animal nutrition. It is also a noble plant; its main physicochemical, nutritional, and nutraceutical characteristics allow nopal utilization in diverse food applications. Over the past decades, the utilize of *Opuntia* for the therapy of metabolic syndrome, which is predominantly linked to diabetes mellitus (DM). Nevertheless, *Opuntia*'s employment has many advantages against disorder, type 2 diabetes, and obesity, among others (Angulo-Bejarano et al., 2019).

*Opuntia* plants are rich in phenolics, vitamins, polyunsaturated fatty acids (PUFA), and amino acids. Many of those compounds were shown to be endowed with biologically relevant activities including antioxidant, anti-inflammatory, hypoglycemic, antimicrobial, and neuroprotective properties (El-Mostafa et al., 2014). The antimicrobial and antioxidant effects of cultivars of cactus pear from Mexico against *Campylobacter jejuni*, *Vibrio cholera*, and eubacteria were studied by Sánchez et al. (2014). Four cultivars were effective against *V. cholera* and *C. perfringens*.

Mucilage is the main complex carbohydrate compound of cladodes. The mucilage may be a viscous liquid produced by the cladodes and consists of different sugars like rhamnose, galacturonic acids, arabinose, galactose, and xylose (Rodríguez-González et al., 2014). This complex polysaccharide has the capacity of absorbing large amounts of water, dissolving, and dispersing in self and forming viscous or gelatinous colloids (Nefzaoui et al., 2007; Yahia et al., 2009). Thus, pectic-derived oligosaccharides and mucilages of *O. ficus-indica* stimulate the expansion of bifidobacteria within the colon of humans and acts as a soluble receptor analog (especially sialic acid) for pathogens and thus have an inhibitory effect on certain pathogenic microorganisms (Magne et al., 2008; Guevara-Arauz et al., 2012).

The cultivars belong to *O. ficus-indica* have the highest economic value in the world (Griffith, 2004). The consumption of *O. ficus-indica* cultivars as a vegetable in the local market is approximately 6.4 kg per capita, and there is an increasing demand for it in the US and Canada, where the product sales ranged between 31 and 2.86 million dollars, respectively (Isela et al., 2017). Apart from bioactive compounds, *O. ficus-indica* has being used for various foodstuffs such as low-calorie marmalade, juices, beverages, jams, and sweeteners (Barba et al., 2017; Aruwa et al., 2018). Also, different parts of the plant used in cosmetics (Galati et al., 2003; Osuna-Martínez et al., 2014), food preservations of meat (Palmeri et al., 2018), high-fat cookies (Msaddak et al., 2015), and sources of bioactive molecules (Msaddak et al., 2017), antimicrobial agents (Guevara-Arauz et al., 2012). This chapter is an upgrade on the bioactive molecule's properties of *Opuntia* spp. as well as their potential as antimicrobial agents and applications in poultry nutrition.

## 2 Scientific Classification, Structure and Chemical Composition

Morphology, scientific classification, and chemical composition and nutritive value of *Opuntia* spp. are illustrated in Fig. 18.1 and Tables 18.1 and 18.2.



Fig. 18.1 *Opuntia littoralis* var. *vaseyi*

Table 18.1 Scientific classification of *Opuntia* spp.<sup>a</sup>

Domain	Eukaryota
Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Order	Caryophyllales
Family	Cactaceae
Subfamily	Opuntioideae
Tribe	Opuntieae
Genus	Opuntia

<sup>a</sup><https://en.wikipedia.org/wiki/Opuntia>

### 3 Extraction and Using of *Opuntia* spp. Products and Byproducts

Nopal is a young pad (cladophyll) of prickly pear (*Opuntia ficus-indica*) which is highly consumed as a vegetable in America, Africa, and Asia. It has been believed that nopal can increase bovine cattle's milk production (Ortiz-Rodríguez et al., 2012). The bacterial count is fewer in fresh cheese during the dry season, and milk production is higher than in other seasons. Feeding of Holstein cows with a diet

**Table 18.2** Chemical composition and nutritive value of *Opuntia*<sup>a</sup>

Nutritional value per 100 g (3.5 oz)	
Energy	41 kcal
Carbohydrates	9.6 g
Dietary fiber	3.6 g
Protein	0.7 g
Fat	0.5 g
Vitamins	Quantity
Vitamin A Equiv.	25 µg
Riboflavin (B2)	0.1 mg
Niacin (B3)	0.5 mg
Vitamin B6	0.1 mg
Folate (B9)	6 µg
Vitamin C	14.0 mg
Vitamin E	0 mg
Minerals	
Calcium	56 mg
Magnesium	85 mg
Iron	0.3 mg
Phosphorus	24 mg
Zinc	0.1 mg
Potassium	220 mg
Water	88 g

<sup>a</sup><https://en.wikipedia.org/wiki/Opuntia>

supplemented with fresh cactus (*O. ficus-indica*) decreases the values of total bacterial count and coliforms group in milk and cheese in the final products (Ortiz-Rodríguez et al., 2013). Ortiz et al. (2011) reported that supplementing mucilage from *O. ficus-indica*, epidermis from *O. ficus-indica*, or dried cladodes from *O. ficus-indica* markedly lowered the counts of coliforms populations in fresh milk. Moreover, liquid mucilage (0.5%) from *O. ficus-indica* and *O. atropes* lowered the mesophilic aerobic bacteria and coliforms counts (Ortiz-Rodríguez et al., 2016).

## 4 *Opuntia* spp. and Their Bioactive Molecules

Among natural colorant sources, cactus (Cactaceae) constitutes one of the medicinal and tinctorial plants. The nutritional value of cactus fruit based on its richness in betalains and phenolics that promote good health through its hypoglycemic, hypolipidemic, and antioxidant properties (Mohamed et al., 2019; Nakashima & Bastos, 2019). In a study on the effect of cactus pear pulp on the quality of fermented rice milk beverage, the count of *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium* BB-12 were above 7 log cfu mL<sup>-1</sup> at the end of storage period.

Sensory evaluation revealed significant differences between control and fermented rice milk beverage samples. The fermented rice milk beverage containing 20% cactus pear pulp had the highest overall acceptability score when compared to control and other treatment samples. It was demonstrated that the addition of cactus pear and physalis pulps to fermented rice milk beverage significantly improved the quality of resultant fermented rice milk beverage.

## 5 *Opuntia* spp. and Their Antimicrobial Molecules

Both academic scientists and the food industry have presented great interest in the *O. ficus-indica* fruits. Various groups of bioactive compounds are detected in *O. ficus-indica*, whole fruit, pulp, flowers, seeds, and peels. They include phenolic acids, flavonoids, anthocyanins, carotenoids, betalains, sterols, lignans, saponins, vitamin E, and vitamin C. The identified *O. ficus-indica* bioactive compounds have demonstrated to be endowed with biological activities such as antioxidant, antimicrobial, anticancer, anti-diabetes mellitus, hypertension, hypercholesterolemic, rheumatic pain, antiulcerogenic activity, gastric mucosa diseases, and asthma (Tahir et al., 2019). The current global trend in the growth of antimicrobial resistance cannot be overemphasized. Sources of antimicrobial compounds are depleting, hence the search for novel antimicrobials from medical plants such as *Opuntia* is of interest (Aremu, Amoo, Ndhkala, Finnie, & Van Staden, 2011); Valtierra-Rodríguez, Heredia, García, & Sánchez, 2010). The antioxidant and antibacterial activities of molecules derived from *Opuntia* spp. are varied depending on the fraction. The unsaponifiable portion has higher biological activities than the glyceridic ones. This portion was more efficient against pathogenic strains of *Escherichia coli*. This fraction has stronger effects of scavenging 2,2-Diphenyl-1-picrylhydrazyl (DPPH-) radical and it is more efficient against bleaching of  $\beta$ -carotene than the saponifiable extract, unlike the experimental results of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) scavenging test (R'bia et al., 2017). The main fatty acids identified in *Opuntia* were linoleic, oleic and palmitic acids. This mean that the unsaponifiable fraction have high content of bioactive compounds (R'bia et al., 2017).

Colorants are used as additives to give food and medicines an attractive appearance and protect their flavor and quality (Delgado-Vargas et al., 2000). There are several types of additives that are permitted in the industry. Nevertheless, several studies have denounced the toxicity of additives based on synthetic colorants. These are added intentionally by food processing, cosmetics, or pharmaceutical industries. Most of these additives are now considered harmless though accused of being sources of allergies, food intolerance and more serious diseases mainly related to immune disorders (Bourrier, 2006; Vojdani & Vojdani, 2015). As a result, consumer aversion to synthetic colorants has increased. For this, the use of natural dyes with limited or no side effects is an alternative to meet consumer demand for healthier food products. According to the presence or absence of nitrogen in their structure, natural bioactive colorants are classified into groups (Delgado-Vargas et al., 2000):



isoprenoid (carotenoids) and benzopyran derivatives (anthocyanin, etc.), and N-heterocyclic compounds (betalains, etc.). These natural pigments are being studied for their possible use as antimicrobial, anti-inflammatory, antioxidant, and anti-cancer agents. Indeed, being antioxidants, they protect the body against damage caused by oxidative stress which is the first step in the genesis of chronic diseases (Melgar et al., 2017; Rahimi et al., 2018). Betalains are of growing interest as a substitute for synthetic colorants for their evolutionary origin and role in stress tolerance. These nitrogen compounds can be classified into red betacyanins and yellow betaxanthins (Chauhan et al., 2013). Unlike other natural red pigments, betalains are stable over a wide pH ranges (4–7). This property makes them ideal pigments with low acidity (Cejudo-Bastante et al., 2016). Similarly, phenolic compounds are also used as additives. Phenolics can scavenge free radicals and enhance natural defenses by protecting tissue constituents (lipids and other macromolecules) against oxidative stress.

## 6 *Opuntia* spp. and Their Antimicrobial Profile and Genotypes

Independent studies either for genetic diversity (Valadez-Moctezuma et al., 2014; Zoghلامي et al., 2007) or chemical profile of cactus (Cayuela et al., 2018; Albano et al., 2015) were undertaken. The association of the molecular approach with the chemical characterization of *Opuntia* species is important. The principal component analysis (PCA) of the antimicrobial profile of *Opuntia* genotypes revealed two distinct groups: the primary one included *O. ficus-indica* and *O. humifusa* species suggesting an analogous antimicrobial potential. While the antimicrobial activity of *O. engelmannii* species has been shown to be clearly distinct from the opposite species as shown by PCA (Salem et al., 2020). The assessment of the genetic diversity of natural resources may be a prerequisite for the definition of the latest strategies of management or genetic improvement. Genetic markers of macromolecule types, whose expression is independent of the environment, may be required to characterize populations and assess their genetic diversity at intra- and inter-population levels using single-sequence repeat (SSR) markers. These latter were used for phylogenetic studies, assessment of genetic variability, and genome mapping. Thus, specific alleles were recorded and proved to be potent for genotyping studied cultivars. Photometric quantification of betalains and phenolics showed an interspecific variation across *Opuntia* species. *O. ficus-indica* fruits showed the highest betalain [betaxanthins; 843.67 and betacyanins; 1400 mg/100 g dry weight (DW)] and phenolics contents. Reversed-phase high-performance liquid chromatography analysis showed that the variation of particular phenolics profile was affected by interspecific and genetic factors. Isorhamnetin-*O*-(di-deoxyhexosyl-hexoside) was the most important compound and its content varied per *Opuntia* species, while catechol was the predominant phenolic compound in *O. humifusa*

with 1.88  $\mu\text{g/g}$  DW. Concerning cactus species, *Opuntia* colorants exhibited a potent antiradical activity [half maximal inhibitory concentration ( $\text{IC}_{50}$ ) up to 1  $\mu\text{g/mL}$ ]. *Opuntia* species were effective against Gram-positive and Gram-negative bacterial strains [inhibition zone (IZ) up to 27 mm] (Salem et al., 2020). High genetic diversity within *Opuntia* genotypes supported SSR markers was revealed. The unweighted pair group method with arithmetic mean dendrogram and principal coordinate analysis supported natural pigments and antimicrobial profiles. The correlation approach proved the presence of a probably metabolic relationship between genetic markers, pigments, and their biological activities. A possible association between the molecular approach and metabolic profile analysis of *Opuntia* allows tracing the connection among species for its genetic conservation (Salem et al., 2020). Cactus extracts, of the chosen species, were screened for their possible antimicrobial activity against pathogenic bacteria, using the solid disc diffusion method. All extracts were active against a minimum of one in each of the bacterial strains tested. The antibacterial potential was addicted to tested *Opuntia* species. Among them, *O. ficus-indica* was the foremost effective one against Gram-positive and Gram-negative bacteria with an IZ starting from 10 to 27 mm. Additionally, *O. engelmannii* and *O. humifusa* extracts showed reduced antibacterial activity. This difference within the antibacterial potential between species may be due to the variation of pigment contents. In fact, *O. ficus-indica* extract was characterized by its richness in phenolic compounds, especially isorhamnetin (Blando et al., 2019). Similarly, other studies have emphasized the antimicrobial effect of betalains. Tenore et al. (2012), and Velićanski et al. (2011) showed the broad antimicrobial spectrum of betacyanins in red pitahaya (*Hylocereus undatus*) and beetroot (*Beta vulgaris*). Among the tested Gram-positive strains, *Staphylococcus aureus* was the foremost sensitive and *O. ficus-indica* extract was the foremost effective against this pathogenic strain with an IZ value of 27 mm. Since this strain is the commonest agent of food-borne diseases (Blando et al., 2019), the result proves the employment of *Opuntia* as a promising source of natural dye for food industry usage. Concerning the Gram-negative bacteria, *Salmonella enteritidis* DMB 560 was the foremost pathogenic resistant bacteria with an IZ value of 10 mm. This might be associated with their great ability to develop resistance to several antimicrobial agents. However, it should be noted, per our knowledge, there are a few studies on the antimicrobial activity of natural dyes from cactus species.

## 7 *Opuntia* spp. and Their Role in Poultry Production

In Cobb broiler diets, Badr et al. (2019) investigated the influences of the replacement of powder of *Opuntia ficus indica* peels (PPP) at various levels (0, 5, 10, and 15%) with yellow corn grain for six weeks on meat quality. The authors reported that feed conversion ratio, live body weight, and feed intake significantly increased using PPP. Marked variations were observed for serum total globulin and serum

total protein in case of PPP compared to the control. Higher degrees of color, taste, texture and odor (aroma) were observed in broiler supplemented with diet contained 15% PPP. The highest net protein utilization, true-digestibility and biological value in case of PPP was attributed to casein in PPP, which is a protein source with high digestibility. In Ross-308 days-old male chicks, PPP decreased some biochemical parameters in blood, such as plasma uremia, glucose, triglycerides, and cholesterol (Moula et al., 2019). Mtambo et al. (1999) evaluated therapeutic and prophylactic efficiency of *Opuntia vulgaris* (prickly pear) against Newcastle disease (ND) in domestic fowl. The consequences suggested that there was no therapeutic or prophylactic value of the plant extract against ND. Moreover, the plant extract revealed a negative influences on body weights in birds with ND. Shah et al. (2016) evaluated the protective effect of *Opuntia elatior* fruit juice against lead acetate toxicity in broiler chickens. Lead acetate was administered at dose rate of 500 ppm in feed, while *Opuntia elatior* fruit juice (3 mL/kg, P.O) was administrated for consecutive 21 days in chickens. There was non-marked reduction in body weight gain and feed intake in birds of lead acetate treated with *Opuntia elatior* fruit juice group. No appreciable histopathological lesions were observed in the spleen and heart of birds in *Opuntia elatior* juice treated groups.

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# Chapter 19

## Genus *Opuntia*: A Golden Source of Compounds with Anti-Inflammatory Potential



Fadia S. Youssef

**Abstract** Genus *Opuntia*, which is also termed by cactus pears or prickly pears, belongs to Cactaceae, the cactus family. The genus name is derived from the Ancient Greek city of Opus. It can perfectly serve as an immense source of compounds offering a plethora of biological activities. Cactus pear is a food showing nutraceutical and functional benefit that ultimately reflects its health-promoting potential manifested by its antioxidant, analgesic, anti-inflammatory, anti-ulcerogenic, anti-hyperglycaemic activities, in addition to its anti-atherogenic and cholesterol-lowering potential. The cactus pear's beneficial value is due to its richness with several compounds as lipids, vitamins, amino acids, minerals, organic acids, carotenoids, and phenolic compounds. Regarding the anti-inflammatory activity, many *Opuntia* species revealed a potent anti-inflammatory potential *via* an effective suppression of inducible nitric oxide synthetase (iNOS), interleukin-6 (IL-6), tumor necrosis factor (TNF- $\alpha$ ), cyclooxygenases, and 5-lipoxygenase (5-LOX) as well. In this chapter, the *Opuntia* species' role to combat inflammation and its consequences will be comprehensively highlighted to offer a guide for those interested in *Opuntia* in their field.

**Keywords** Secondary metabolites · Nitric oxide synthetase (iNOS) · Tumor necrosis factor (TNF- $\alpha$ ) · Interleukin-6 (IL-6) · 5-lipoxygenase (5-LOX)

## 1 Introduction

Natural products derived from plant origin act as everlasting sources of secondary metabolites that displayed a wide range of therapeutic activities that undoubtedly alleviate an unlimited number of diseases (Ashour et al., 2018). Drugs derived from

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the natural origin are relatively safer and lower in price relative to drug entities obtained from the synthetic origin (Talaat et al., 2018). Moreover, they are highly acceptable among a people all over the globe (Janibekov et al., 2018).

Inflammation is a severe pathophysiological cascade triggered by the number of molecular signals caused by macrophages, leukocyte infiltration, and mast cells (Labib et al., 2017; Labib et al., 2018). This consequently forms edema due to protein extravasation, the infiltration of leukocytes, and fluid accumulation. Besides, untreated inflammation that exists chronically for a prolonged period adversely affects tissue regeneration, which ultimately triggers various dangerous and life-threatening conditions such as diabetes, cancer, autoimmune and neurodegenerative disorders (Ashour et al., 2018).

Nowadays, there is an outstanding effort exerted worldwide to discover new drug entities combating inflammation with no adverse effects provoked by the commonly known synthetic anti-inflammatory agents, particularly the non-steroidal anti-inflammatory drugs NSAIDs. Natural metabolites, particularly those derived from food, are highly welcomed by populations both in developed and developing countries. Those showing anti-inflammatory activity could perfectly be incorporated in pharmaceutical preparations and cosmetics, serving beneficial multipurpose activity concerning synthetic agents (Ashour et al., 2018).

Genus *Opuntia* is a xerophyte belonging to the family Cactaceae and comprises about 200–300 species, mainly planted in arid and semi-arid zones. The remarkable genetic variation among the different species resulted in its cultivation in different climates. Traditionally, cactus plants act as fruits and vegetable sources and could be employed for therapeutic and cosmetic purposes. Besides, it can act as a building material, forage, and natural colorants as well. *Opuntia* fruits, commonly known as cactus pears or prickly pears, are locally used as fresh fruit, juice, and sweets. Studies declared that many *Opuntia* species and the fruit juice, in particular, are rich in many functional ingredients that could counteract many health disorders (Stintzing & Carle, 2005). In this chapter, a highlight will be shed on the anti-inflammatory potential of different *Opuntia* species and their secondary metabolites. The activity is attributed to afford a guide for those interested in *Opuntia* in their research. Different *Opuntia* species possessing anti-inflammatory activity are illustrated in Fig. 19.1.

## **2 Anti-Inflammatory Activity of Different *Opuntia* Species and their Bioactive Compounds Arranged in Alphabetical Order**

Representatives of secondary metabolites derived from different *Opuntia* species with anti-inflammatory activity are illustrated in Fig. 19.2.





*Opuntia dillenii*



*Opuntia elatior*



*Opuntia ficus-indica*



*Opuntia humifusa*



*Opuntia macrorhiza*



*Opuntia microdasys* var. *rufida*

Fig. 19.1 Different *Opuntia* species possessing anti-inflammatory activity

## 2.1 *O. dillenii*

*Opuntia dillenii* aqueous ethanol extract obtained from its fresh cladodes displayed considerable anti-inflammatory potential. From this bioactive extract, two new compounds with  $\alpha$ -pyrone skeleton were isolated: opuntioside II and opuntioside III that were comprehensively elucidated based upon their chemical characters and physicochemical evidence (Qiu et al., 2007).

## 2.2 *O. elatior*

*Opuntia elatior* fruits are highly popular in traditional medicine by possessing a significant role in alleviating many diseases comprising inflammatory disorders, obesity, diabetes, asthma, and anemia. These pronounced activities were mainly attributed to betanin (1) (Fig. 19.2) isolated from the fruits and revealed a significant anti-inflammatory and antioxidant activity (Sutariya & Saraf, 2017).

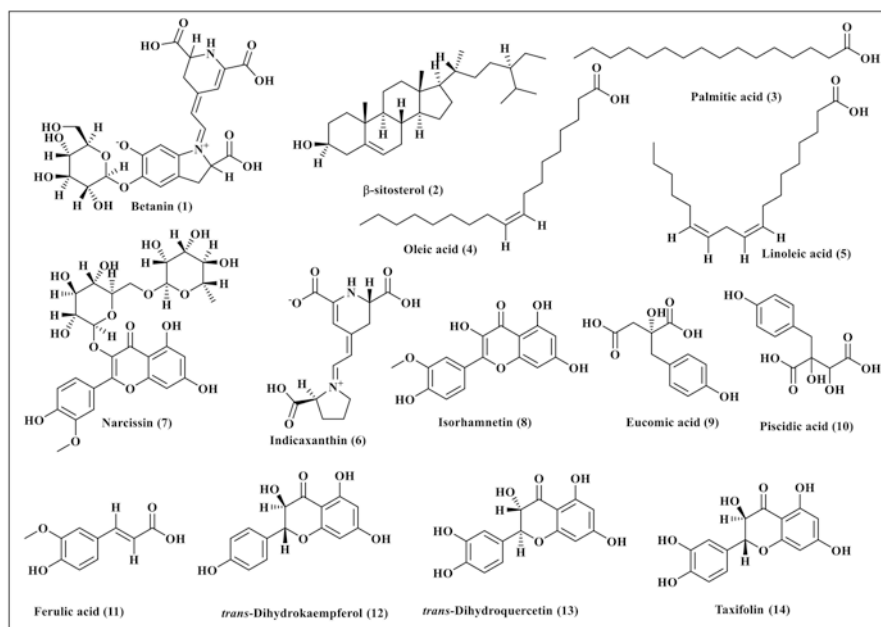


Fig. 19.2 Secondary metabolites derived from *Opuntia* species with anti-inflammatory activity

### 2.3 *O. ficus-indica*

*Opuntia ficus-indica* fruits were adopted in Saudi Arabia as a popular functional food showing anti-inflammatory potential. The seed oil was also proved to possess an anti-inflammatory effect *via in vivo* study employing croton oil-induced ear edema and carrageenan-induced paw edema models at two doses, which are 100 and 200 mg/kg. The two doses effectively prohibited edema formation in a dose-dependent manner estimated at 46% and 62%, respectively, in addition to reducing prostaglandin (PGE<sub>2</sub>) concentration by 54% and 67%, respectively. Regarding the croton oil-induced ear edema model, the two doses reduced the edematous ear by 20% and 33% in addition to reducing myeloperoxidase (MPO) activity by a value estimated by 54% and 62%, respectively. These were further supported by histopathological studies that revealed significant amelioration in the inflammation using the seed oil. This could be attributed to the presence of  $\beta$ -sitosterol (2) (24.9%), palmitic acid (3) (10.6%), oleic acid (4) (8.16%) as well as linoleic acid (5) (5.9%) that represent the chief components in the seed oil (Koshak et al., 2020). It is noteworthy to highlight that betacyanin obtained from prickly pear fruit serves as a natural dye with a potent anti-inflammatory effect (Esatbeyoglu et al., 2015).

Another study was conducted on indicaxanthin (6), one of *Opuntia ficus indica* major compounds, and showed that it effectively prohibits oxidized LDL-mediated human endothelial cell dysfunction *via* its redox-modulating anti-inflammatory effect manifested by a significant NF- $\kappa$ B inhibition. It is well-known that oxidized LDL (low-density lipoproteins) has a crucial role in the pathogenesis observed in atherosclerosis *via* activation of inflammatory signaling steps resulting in endothelial dysfunction senescence. Pretreatment with indicaxanthin notably inhibited oxLDL-induced cytotoxicity with concomitant elevation in ICAM-1 (Intercellular Adhesion Molecule 1), VCAM-1 (Vascular cell adhesion protein 1), and ELAM-1 (Endothelial leukocyte adhesion molecule 1) levels in addition to causing significant reduction in ABC-A1 (ATP-binding cassette transporter) levels of both protein and mRNA levels (Attanzio et al., 2019). Besides, indicaxanthin (6) displayed a considerable role in modulating arachidonate metabolism and the synthesis of prostaglandin synthesis *via* lipid peroxide generation in LPS-stimulated macrophages in a concentration-dependent manner. This was achieved *via* prevention of nuclear factor- $\kappa$ B (NF- $\kappa$ B) expression as well as the prohibition of PGE<sub>2</sub> synthase-1 (mPGES-1) overexpression; meanwhile, it triggers PGD<sub>2</sub> synthase (H-PGDS) as well as cyclo-oxygenase-2 (COX-2) up-regulation with the ultimate production of anti-inflammatory cyclopentenone (Allegra et al., 2014a). Besides, indicaxanthin prevents NADPH oxidase's activation and the release of inflammatory mediators, which are NF- $\kappa$ B-dependent, with a concomitant reduction in epithelial permeability cells in Caco-2 cells (human intestinal epithelial cell line) stimulated by IL-1 $\beta$ . It effectively prohibited the formation of ROS as well as pro-inflammatory cytokines represented by IL-8, IL-6 in addition to inhibiting PGE<sub>2</sub> and NO with concomitant loss of thiols in a dose-dependent behavior (Tesoriere et al., 2014). Indicaxanthin obtained from *Opuntia ficus-indica* edible fruits additionally showed

anti-inflammatory activity in a rat model adopting carrageenin induced inflammation in a rat model. Indicaxanthin administered orally at doses of 0.5, 1, or 2  $\mu\text{mol/kg}$  before the administration of carrageenin reduced the volume of exudate by 70% in a dose-dependent manner concomitant reduction in the leukocytes number exist in the pleural cavity by 95%. Additionally, it inhibited the release of IL-1 $\beta$ , TNF- $\alpha$ , NO, PGE2 by 53.6, 71.1, 67.7, 91.4%, respectively. It concomitantly reduced iNOS, COX-2, IL-1 $\beta$  and TNF- $\alpha$ , by 75.2, 87.7, 34.5 and 81.6%, respectively with reduction in mRNA and protein expression in the treated leukocytes. Besides, it prohibited the generation of NF- $\kappa\text{B}$ , a key transcription factor in the whole inflammatory cascade both in a time- and dose-dependent manner (Allegra et al., 2014b; Gómez-Maqueo et al., 2019b).

Narcissin (7) constitutes the chief flavonoid glycoside in *Opuntia ficus indica* fruits. Upon exposure to hydrolysis by enzymes, narcissin yielded an aglycone that effectively displayed estrogen receptor  $\beta$ -selective transcriptional activity with the concomitant prohibition of a nuclear factor- $\kappa\text{B}$  signaling pathway using a cell line. Besides, it effectively inhibited the catalysis during the transformation of prostaglandin H2 to prostaglandin D2 with inflammatory potential (Jeong et al., 2016). Additionally, phenolic acids and flavonoids that mainly exist in the flowers of *Opuntia ficus-indica*, as revealed from LC-MS analyses, displayed a potent anti-inflammatory activity. This was evidenced by the results obtained from the carrageenan-induced rat paw edema model. Phenolic acids and flavonoids rich fraction considerably prohibited inflammation *via* decreasing malondialdehyde level, increasing superoxide dismutase, catalase, and reduced glutathione (Ammar et al., 2018).

Isorhamnetin (8) conjugates were released upon enzymatic hydrolysis of *Opuntia ficus-indica* fruit using supercritical CO<sub>2</sub>, which differ by changing hydrolysis conditions to give isorhamnetin-3-*O*-glucosyl-rhamnoside, isorhamnetin-3-*O*-glucosyl-rhamnosyl-pentoside as well as isorhamnetin-3-*O*-glucosyl-rhamnosyl-rhamnoside that act as anti-inflammatory agents *via* suppressing nitric oxide production with varying degrees. Noteworthy is that the sample containing a higher percentage of isorhamnetin-3-*O*-glucosyl-rhamnosyl-rhamnoside revealed the most significant inhibition to nitric oxide generation represented by 71.6% with undoubtedly higher anti-inflammatory activity (Antunes-Ricardo et al., 2018). An additional study was carried on two isolated isorhamnetin glycosides isolated from *Opuntia ficus-indica* cladodes isorhamnetin-3-*O*-glucosyl-rhamnoside and isorhamnetin-3-*O*-glucosyl-rhamnosyl-rhamnoside. Isorhamnetin-3-*O*-glucosyl-rhamnosyl-rhamnoside showed the highest potential to reduce cell infiltration, particularly neutrophils, with 51.8% reduction. Meanwhile, the total seed extract and compound effectively prohibited nitric oxide production with 77.2 and 81.4% inhibition, respectively, in addition to prohibiting cyclooxygenase-2 activity with 77.7 and 76.3% inhibition. Moreover, the seed extract revealed the most significant inhibition to interleukin (IL)-6 and of tumor necrosis factor (TNF)- $\alpha$  with 53 and 85.2% inhibition, respectively (Antunes-Ricardo et al., 2015).

*Opuntia ficus-indica* flowers methanol extract showed a potent anti-inflammatory activity estimated at 53.8% inhibition in the inflammation attributed to its isolated

compound, isorhamnetin 3-*O*-robinobioside that produced 77.5% inhibition in the inflammation (Seddik Ameur et al., 2016). Additional research showed that fermentation of cactus cladodes obtained from *Opuntia ficus-indica* using lactic acid-producing bacteria produces flavonoid derivatives with higher anti-inflammatory activity as secondary metabolites induced and modified in the plant (Filannino et al., 2016).

An additional study showed that *Opuntia ficus-indica* seed alleviates liver steatosis and inflammation *via* controlling *de novo* lipogenesis and macrophage polarization *versus* nonalcoholic liver steatosis using normal or a high-fat diet animal model. Treatment using the seed significantly reduced HFD-stimulated elevation in liver lipid content as well as hepatocellular damage. It effectively raises sterol regulatory element-binding protein 1 as well as carbohydrate-responsive element-binding protein expression with a concomitant reduction in carnitine palmitoyltransferase 1A. Besides, it reduced the expression of peroxisome proliferator-activated receptor  $\gamma$ . In addition, it stimulated hepatic M2 macrophage polarization evidenced by M1 marker genes attenuation and M2 marker genes stimulation. Furthermore, it reduced toll-like receptor 4 expressions together with tumor necrosis factor  $\alpha$ , interleukin 6, TIR-domain-containing adapter inducing interferon  $\beta$ , nuclear factor  $\kappa$ B, and interferon  $\beta$  levels as well (Kang et al., 2016).

Prickly pear seed hydroethanolic and water extracts administered orally at a dose of 500 mg/kg were examined for their anti-inflammatory activity *in vivo* using the carrageenan-induced rat paw edema model. The seed hydroethanolic displayed a potent reduction in edema caused by carrageenan-induced rat paw edema estimated by 44% and 50% after 90 min and 3 h; respectively. The water extract decreased the elevation in paw volume by 25% relative to the control group. The observed anti-inflammatory activity is mainly attributed to the extract's richness with alkaloids, flavonoids, sterols, and tannins (Benattia et al., 2017).

The flavonoid-rich fraction obtained from the juice of *Opuntia ficus indica* comprising mainly of isorhamnetin 3-*O*-rutinoside, isorhamnetin 3-*O*-rhamnoserutinoside, eucomic (9), piscidic (10), and ferulic (11) acids revealed potent antioxidant as well as anti-inflammatory potential counteracting intestinal inflammation. Pre-incubation of the flavonoid-rich fraction within Caco-2 cells effectively reduced radicals production induced by stressors, declaring its secondary metabolites' ability to work at the intracellular level. This was evidenced by an effective prohibition on generating H<sub>2</sub>O<sub>2</sub>-induced radicals in intestinal epithelial cells' surrounding environment with a concomitant reduction in IL-8 secretion, I $\kappa$ B $\alpha$  degradation, NO, and TNF- $\alpha$  expression (Matias et al., 2014).

The flowers of *Opuntia ficus indica* are a rich source of phenolics, hydroxycinnamic acids, procyanidins, and flavonoids that increased obviously upon extraction using 80% acetone in the water at 80 °C that revealed a potent anti-inflammatory activity. Undoubtedly, cactus flowers could afford bioactive ingredients serving as anti-inflammatory agents that could be incorporated in foods, cosmetics, and pharmaceutical products (Benayad et al., 2014).

*Opuntia ficus-indica* crushed pads and fruits were found to possess considerable components manifested by minerals such as calcium ranging between 4391.2 to

2086.9 mg %, potassium with amounts of 1932.1 to 2608.7 mg % in addition to magnesium that ranges between 800.6 to 1984.8 mg %. Besides, amino acids also exist with glutamic acid represents the major compound forming 16.3% of total amino acids present in the fruit and 25.2% in the pad. Dihydroflavonols are represented by *trans*-dihydrokaempferol (12) and (+)-*trans*-dihydroquercetin (13). Citric acid methyl esters obtained from fruits displayed effective inhibition *versus* monoamine oxidase-B. Besides, *Opuntia ficus-indica* acts as a rich source of food and medicinal components that revealed antimicrobial activity (Lee et al., 2005).

Moreover, the ethanol extract obtained from the stems of cactus stem affords a potent anti-inflammatory agent. By successive fractionation using different solvents such as *n*-hexane, ethyl acetate, and *n*-butanol, *n*-hexane exhibited the most potent anti-inflammatory activity employing the carrageenan-induced paw edema model (Park et al., 1998). Additionally, the submission of the prickly pears to an elevated hydrostatic pressure by 100, 350, and 600 MPa at a temperature ranging between 17 and 34 °C for 5 min resulted in an observable enhancement in the extractability of bioactive compounds represented by phenolics, betalains, and ascorbic acid as revealed from high-performance liquid chromatography and spectrophotometry analyses of peels and in pulps as well. This consequently reflected the pears' biological activity's enhancement manifested by increasing its anti-inflammatory activity (Gómez-Maqueo et al., 2019a).

## 2.4 *O. humifusa*

*Opuntia humifusa* cladodes are a rich source of megastigmane derivatives that showed a potent nitric oxide inhibitory potential in lipopolysaccharide (LPS)-induced RAW 264.7 cells. This is probably due to their inhibition in the expression of iNOS (Jo et al., 2020). Taxifolin (14) isolated from *Opuntia humifusa* leaves was investigated for its anti-inflammatory activity in lipopolysaccharide-stimulated RAW 264.7 murine macrophages. Taxifolin displayed a potent inhibition on LPS-induced tumor necrosis factor- $\alpha$  and interleukin-6 generation, as revealed by cytokine assay. Additionally, it effectively reduced the expression of cyclooxygenase-2, as evidenced by western blot analysis. Meanwhile, it showed no cytotoxicity on RAW 264.7 cells at a concentration of 500  $\mu$ M that indicates its safety. A study conducted on the fermented product of *Opuntia humifusa* extract showed its potent anti-inflammatory activity evidenced by its significant scavenging activity of nitric oxide associated with inflammation and thus be incorporated in many cosmetic and pharmaceutical preparations (Kim et al., 2015). Besides, an additional study performed on *Opuntia humifusa* fruit extracts revealed that chromen-based compounds represented by isorhamnetin-3-O- $\beta$ -D-galactosyl-4'-O- $\beta$ -D-glucoside and isorhamnetin-3-O- $\beta$ -D-glucosyl-4'-O- $\beta$ -D-glucoside are highly effective as anti-inflammatory agents existing in functional food and with no apparent adverse effects or cytotoxicity (Cho et al., 2006). The roots extract of *Opuntia humifusa* also

revealed promising anti-inflammatory, anti-oxidative and anti-tumor activities (Cho et al., 2006).

The anti-inflammatory activity of *Opuntia humifusa* was revealed to contribute to the alleviation of acute pancreatitis induced by caerulein. Results showed that intraperitoneal administration of *Opuntia humifusa* at different doses 100, 250, or 500 mg/kg effectively ameliorated acute pancreatitis evidenced by histopathology of the pancreas as well as the inhibition of neutrophil infiltration, proinflammatory cytokine expression represented by IL- 1, IL- 6, as well as TNF-  $\alpha$  and cell death comprising apoptosis and necrosis with the concomitant prohibition of c-Jun N-terminal kinases (Choi et al., 2014).

An additional study was carried on the different extracts of *Opuntia humifusa* stem screening their anti-inflammatory effect in two cancer cells, namely breast cancer (MCF7) and human colon cancer (SW480). Results clarified that the ethyl acetate fraction triggers a significant inhibition in the inflammation pathways. A notable down-regulation manifested this in the expression of inflammatory mediators represented by cyclooxygenase-2 (COX2) as well as inducible nitric oxide synthase (iNOS) in human colon cancer (SW480) but not in namely breast cancer (MCF7) (Kim et al., 2013).

The ethyl acetate and chloroform fractions of *Opuntia humifusa* revealed potent and anti-inflammatory effects in lipopolysaccharide (LPS)-activated RAW264.7 cells evidenced by lowering iNOS expression as well as the prohibition of inflammatory cytokines represented by IL-1 $\beta$  that is mainly attributed to the presence of quercetin as a chief component in the former extract (Cho et al., 2006).

## 2.5 *O. macrorhiza*

*Opuntia macrorhiza* seed oil was chemically analyzed and biologically assessed for different activities. The oil is highly suitable to be employed as edible oil due to its high stability versus oxidation, low acid value manifested by high peroxide level, richness with the saponifiable matter, and unsaturated fatty acids. This was positively reflected on its biological behavior where the seed oil revealed potent anti-inflammatory, antioxidant,  $\alpha$ -glucosidase inhibitory activity, and antimicrobial effects with no evidence for the occurrence of acute toxicity when tested *in vivo*, indicating its safety (Chahdoura et al., 2017).

## 2.6 *O. microdasys*

Pectin isolated from the peel and pulp of *Opuntia microdasys* var. *rufida* cladodes was analyzed using various chromatographic techniques coupled with spectrometric techniques. Different pectin analyses revealed that uronic acids and neutral sugars such as arabinose, galactose, rhamnose, and mannose mainly exist in the oil with

a molecular weight ranging between 2,180,000 and 4,920,000 g/mol. Besides, upon intraperitoneal injection of the pectin extracted from the peel and pulp at doses 50–100 mg/kg, considerable anti-inflammatory activity was observed in both xylene-induced ear edema in mice and carrageenan-induced paw edema in rats in a dose-dependent manner (Jouini et al., 2018). Besides, the aqueous flower extract of *Opuntia microdasys* effectively alleviated inflammation triggered by carrageenan in animal models, evidenced by the reduction of carrageenan-induced paw edema by 70.1% (Chahdoura et al., 2017).

## 2.7 *O. stricta*

The ethyl acetate extract obtained from *Opuntia stricta* flower revealed potent anti-inflammatory activity in human peripheral blood mononuclear cells inflammation triggered by lipopolysaccharide evidenced by inhibition of nitric oxide production and maintaining cell viability (Izuegbuna et al., 2019). *Opuntia stricta* cladodes extracted by different solvents represented by water, ethanol, and acetone revealed notable anti-inflammatory activity using cell-based assays employing RAW 264.7 cells. They revealed considerable amounts of vitamins A, C, and E and phenolic compounds to which the activity is attributed (Izuegbuna et al., 2019).

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## Chapter 20

# *Opuntia* spp. Benefits in Chronic Diseases



Yasmina M. Abd-Elhakim and Adham A. Al-Sagheer

**Abstract** There is a growing trend for the use of nutritional and medicinal natural compounds to manage chronic diseases. In particular, flavonoids have gained much interest because of their regulation effects on oxidative stress and inflammatory events linked to chronic disorders initiation and progression, including metabolic, neurodegenerative, and neoplastic diseases. For centuries, *Opuntia* species have served as food and traditional folk medicine for their nutritional features and their benefits in chronic conditions like obesity, cancer, diabetes, and cardiovascular diseases. The plant is globally distributed and has high economic potential. The different parts of the plant, including pear, cladodes, roots, and seeds, have favorable biological activities. The plant parts are rich in phenolic acids, biopeptides, ascorbate, flavonoids, betalains, carotenoids, and soluble fibers, which possess favorable pharmacological activities promising agent in the development of drugs for chronic disease intervention. In this chapter, the role of the most commonly studied *Opuntia* spp. and its bioactives in the management of chronic diseases has been described. Also, a special focusing has been paid on the safety aspects and potential toxicities, which should be considered to achieve the maximum benefit of the *Opuntia* spp. in chronic disease therapies.

**Keywords** Indicaxanthin · Chronic wounds · C-reactive peptide · Chronic diseases · Obesity · Diabetes · Cancer · Safety · Arthritis

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## Abbreviations

AD	Alzheimer's disease
BBB	Blood–brain barrier
CRP	C-reactive peptide
CVD	Cardiovascular disease
DM	Diabetes mellitus
HA	Hyaluronic acid
HDL-C	High-density lipoprotein cholesterol
HFD	High-fat diet
IBDs	Inflammatory bowel diseases
LDL-C	low-density lipoprotein cholesterol
NF- $\kappa$ B	Nuclear factor kappa B
Nrf2	Transcription factor NF-E2-related factor 2.
OGTT	Oral glucose tolerance test
PD	Parkinson's disease
ROS	Reactive oxygen species
STZ	Streptozotocin
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TG	Triglycerides
TNF- $\alpha$	Tumor necrosis factor- $\alpha$
VLDL	Very-low-density lipoprotein cholesterol
WHO	World Health Organization

## 1 Introduction and Objectives

Chronic diseases are not degenerative or infectious, with morbid conditions continue for no less than 3 months (Bernell & Howard, 2016). The World Health Organization (WHO) reported that chronic diseases result in 38 million deaths per year, representing 63% of all deaths worldwide (World Health Organization, 2013, 2018). The seven most prevailing chronic diseases are diabetes mellitus (DM), cancer, hypertension, heart disease, stroke, mental illness, and pulmonary illnesses (Bernell & Howard, 2016).

Medicinal plants have become an essential component of conventional medicine in the world, in particular, as their beneficial effects have been approved by scientific evidence as alternative therapies in different conditions (Abd-Elhakim, et al., 2020a, b; El-Rahman et al., 2020; El-Saber Batiha et al., 2020; Hashem et al., 2020). Compared to drugs or chemical-based therapies, medicinal plants, besides their predicted positive effects, are more affordable and have fewer adverse effects (Nair et al., 2013). The use of natural compounds from medicinal plants to treat chronic diseases has, thus, become a notable trend in clinical studies (Owona et al., 2020).

The *Opuntia* plant is one of the most popular medicinal treatments for chronic diseases and food sources primarily because it has nutritional and biological properties (Aruwa et al., 2018). The *Opuntia* species has a worldwide distribution and significant economic potential (Moussa-Ayoub et al., 2014). The beneficial advantages of all *Opuntia* products, including cladodes, seeds, roots, juice, and pear, are primarily attributable to the high content of phenolic acids, antioxidants (ascorbate or flavonoids), and pigments (betalains, carotenoids) (Gaballah et al., 2016). These and other phytochemicals, including coumarins, sterols, esters, alkaloids, and terpenoids, have various pharmacologically and physiologically important roles like hypoglycaemic (Paiz et al., 2010), antioxidant (Osorio-Esquivel et al., 2011), diuretic (Galati et al., 2002), anti-inflammatory (Benayad et al., 2014), anti-atherosclerotic (Wang et al., 2015), and anti-angiogenic (Sohail et al., 2014) together with high wound healing capacity (Ammar et al., 2015). The former *Opuntia* spp. biological properties strongly nominate it as a candidate nutraceutical product in managing chronic diseases, mainly DM, cardiovascular diseases (CVD), obesity, and cancer (Del Socorro Santos Díaz et al., 2017). This chapter offers an overview of the protective or therapeutic role of different *Opuntia* spp. in managing chronic diseases. Additionally, particular emphasis was paid on the safety aspects and potential toxicities that should be considered with their use.

## 2 Potential Role of *Opuntia* spp. as Preventive and Therapeutic Agents for Chronic Diseases

### 2.1 *Diabetes Mellitus (DM)*

DM is a metabolic condition characterized by a persistent rise in blood glucose since insulin synthesis and/or activity have been compromised (Kerner & Brückel, 2014). The total number of patients with DM in 2017 was estimated at around 415 million, which is expected to rise to 642 million by 2040 (Ogurtsova et al., 2017). Today, there is a global tendency towards the preventive and therapeutic treatment of DM *via* medicinal plant use (Shabab et al., 2021). Several studies from diabetic patients and animals have reported *Opuntia* antihyperglycemic and antihyperinemic properties (Del Socorro Santos Díaz et al., 2017).

Ibanez-Camacho et al. (1983) studied the *O. streptacantha* sap hypoglycaemic effect in different animals and experiment conditions. They noticed that the sap hypoglycaemic activity had not been detected with pancreatectomized or normoglycaemic animals but only under moderate blood sugar rise. The impact of saps obtained from *O. streptacantha* through the year was studied in rabbits using *in vivo* hypoglycaemic bioassays to determine if biological variations were seasonal. The sap has been reported to have constant activity, indicating that the plant can be used by collecting the sap any time of the year (Meckes-Lozoya & Ibáñez-Camacho, 1989).

After heating *Opuntia* extracts, the hypoglycemic efficacy could increase, as Frati-Munari et al. (1988) stated in patients, ingested boiled extracts of *O. streptacantha*. In eight patients with Type 2 DM (T2DM) in a placebo-controlled crossover experiment, after 500 g broiled crude extracts and cactus stems were taken, Roman-Ramos et al. (1995) compared the serum glucose levels to basal ones. No modifications have been made to blood glucose levels using raw extracts. However, the ingestion of the ground plant stems contributed to a 3 h decrease in serum glucose levels compared with basal levels. The authors suggested heat could be required for a hypoglycemic effect.

Frati et al. (1990) provided a good insight on cactus pear cladode's acute hypoglycemic effect. In this study, unprocessed grilled *O. streptacantha* stems were administered to both T2DM and healthy fasting subjects to investigate the short-term influence on blood glucose and insulin level. Blood glucose was reduced in diabetic patients within 60 min, well below the control level, blood insulin was reduced similarly. This decrease in glucose and insulin was not found in healthy individuals. In a related protocol, eight T2DM diabetic subjects and six healthy people orally consumed 500 g broiled *O. streptacantha* stems. The diabetic patients were lower in serum glucose but not in C-reactive peptide (CRP), while no effect in healthy subjects was observed (Frati et al., 1991).

Different *Opuntia* spp. showed hypoglycemic effects in various animal models. For instance, in streptozotocin (STZ)-diabetic rats, *O. fuliginosa* prickly pear extract supplementation lowered glycemia and glycated hemoglobin to the normal values (Trejo-González et al., 1996). Also, in an acute study of STZ-induced diabetic pigs, Laurenz et al. (2003) assessed the *O. lindheimeri* Englem extract antidiabetic prospective at doses of 250 or 500 mg/kg. The authors showed that a dose- and time-dependent lowering of blood glucose were recorded. The hypoglycemic activity was observed in 1 h of the extract dosing, with the highest effects of 4 h post-administration. Likewise, a significant hypoglycemic effect was observed in diabetic mice following *O. ficus-indica* or *O. streptacantha* polysaccharides consumption (Alarcon-Aguilar et al., 2003).

Godard et al. (2010) performed a clinical trial using a product named OpunDia™ comprising a mixture of cladode and fruit extracts of *O. ficus-indica* to assess its acute and chronic hypoglycemic effects in pre-diabetic obese women and men. The authors reported that the blood glucose decreased when the extract was administered earlier oral glucose tolerance test (OGTT), signifying the acute hypoglycemic effects of *Opuntia* spp. in postprandial conditions and the longstanding cactus safety.

Moreover, in STZ-treated rats, *O. humifusa* stems promoted a significant reduction in the blood glucose level (Hahm et al., 2011). Andrade-Cetto and Wiedenfeld (2011) reported that in STZ-treated rats, the *O. streptacantha* extracts do not decrease glycemia but have an antihyperglycemic effect before the OGTT. In normal rats, Butterweck et al. (2011) combined with the ratio of 75:25 and orally examined the possible anti-diabetic effects of stem and fruit. The most important finding was a decrease in blood glucose and/or throughout OGTT and a rise in the amount of basal plasma insulin, which directly affected pancreatic  $\beta$ -cells.

On the other hand, the influence of cladode and fruit skin extract of *O. ficus-indica* on glucose and plasma insulin increases in high-dose carbohydrate intake, before and after exercise, has been assessed by Van Proeyen et al. (2012). The extract induced increased plasma insulin has also been noticed and thus allowed the removal from circulation of the oral glucose load. This drop in blood glucose was more explicit than in a basal state after exercise.

Nuñez-López et al. (2013) reported that *O. ficus-indica* consumption could decrease serum insulin, postprandial blood glucose, and plasma glucose-dependent insulinotropic peptide peaks, in addition to antioxidant activity augmentation in both healthy individuals and T2DM patients. Besides, López-Romero et al. (2014) reported that the *O. ficus-indica* dietary intake in T2DM patients after ingesting a breakfast with high-soy-protein or high-carbohydrate content significantly enhanced the insulin level, glucose-dependent insulinotropic peptide index, postprandial glucose response, and the glucagon-like peptide 1 index.

*O. ficus-indica* seed oil reduced postprandial hyperglycemia in rats that STZ-induced. This effect was also observed in healthy animals when intestine glucose absorption decreased by approximately 25.4% (Berraouan et al., 2014). In alloxan-induced diabetic mice, the *O. ficus-indica* seed oil displayed a hypoglycemic effect together with islets of Langerhans protection against tissue disturbances (Berraouan et al., 2015). Gao et al. (2015) verified that the polysaccharide from *O. dillenii* Haw. fruits markedly improved the pancreatic islet tissue structural integrity in diabetic rats caused by STZ, suggesting its potent hypoglycemic and antioxidant properties. Also, in the study of Leem et al. (2016) in diabetic mice, *O. ficus indica* dried cladodes aqueous extracts displayed a dose-dependent improvement of hyperinsulinemia, hyperglycemia, and glucose tolerance together with increasing of the  $\beta$ -cell mass.

In the alloxan and fructose-induced diabetes rat model, Chahdoura et al. (2017) reported that oral administration of 200 mg/kg of *O. microdasys* flowers aqueous extract for 28 days significantly lowered the glucose levels suggesting its antidiabetic activity. Also, in STZ-induced diabetic rats, Sutariya and Saraf (2017) reported that oral administration of 25, 50, and 100 mg/kg of betanin from *O. elatior* for eight weeks not only reduced blood glucose but likewise attenuated the kidney injury associated with DM.

Bouhrim et al. (2020) showed a significant antidiabetogenic effect of *O. dillenii* seed oil in albino mice by decreasing blood sugar level rise, bodyweight loss, and mortality rate resulted from alloxan. Li et al. (2020) reported that *O. milpa* polysaccharides alleviated alloxan inducing apoptosis of  $\beta$ -cell *via* oxidative stress attenuation and transcription factor NF-E2-related factor 2 (Nrf2) expression upregulating.

Overall, several proposed mechanisms could be underlying the *Opuntia* spp. antidiabetic activity, as demonstrated in Fig. 20.1. The hypoglycemic mechanism evoked by *Opuntia* spp. consumption could be associated with high dietary fiber content like mucilage and pectin (Frati-Munari et al., 1988), which may delay the absorption of glucose by increasing the viscosity of food in the gut (Lopez, 2007; Shapiro & Gong, 2002). The disaccharide hydrolysis has been suggested to elucidate the *Opuntia* spp. hypoglycemic effect through  $\alpha$ -glucosidase inhibitors

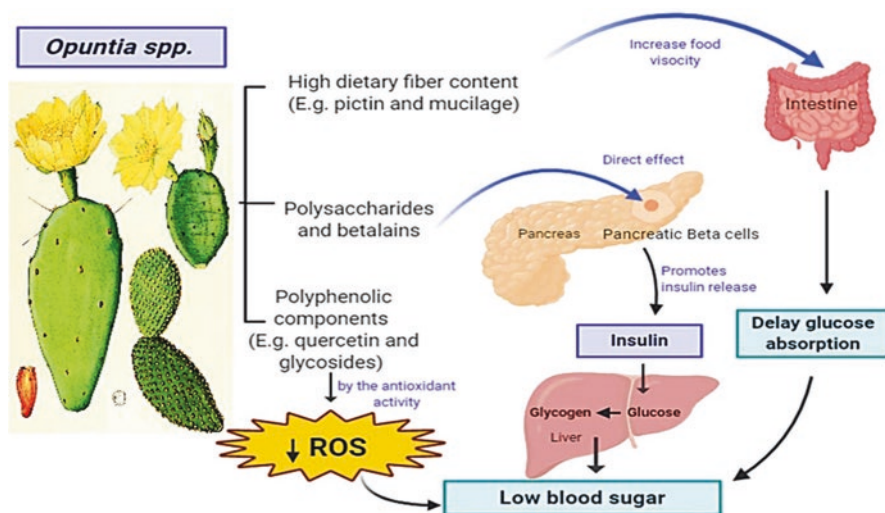


Fig. 20.1 Proposed mechanisms of the antidiabetic activity of *Opuntia* spp.

suppression or a barrier function. Nevertheless, this assumption was not confirmed (Becerra-Jiménez & Andrade-Cetto, 2012). Another postulate is that *Opuntia* spp. induce insulin secretion through the direct effect on pancreatic  $\beta$ -cells (Butterweck et al., 2011), a mechanism also detected in healthy individuals after exercise and implicated in quick resynthesis of glycogen (Deldicque et al., 2013a).

As oxidative stress plays a crucial role in the pathophysiology of T2DM (Giacco & Brownlee, 2010), the *Opuntia* spp. antioxidant properties might play a part in preventing complications of T2DM by counteracting hyperglycemia-induced oxidative stress. In this context, Berraouan et al. (2015) reported that *O. ficus-indica* seed oil augmented the alloxan-induced diabetes development in mice by suppressing the reactive oxygen species (ROS) release. While most attention has so far been put on the antidiabetic effect of prickly pear, primarily on polysaccharides and betalains, the phenolic components may contribute to those effects as well. Habtemariam and Varghese (2014) has reviewed the antidiabetic activity of the phenolic components and reported that compounds such as quercetin and glycosides have in vitro and in vivo antidiabetic effects (Habtemariam, 2011; Habtemariam & Lentini, 2015).

Recently, Gouws et al. (2019) published a systematic review of 335 articles on the anti-diabetic properties or anti-hyperglycemic effects of *Opuntia* spp. and its products. The authors indicated that significant variations exist in the activities between cacti products and components. For instance, cladode and some *Opuntia* spp. products showed substantial reductions in serum glucose and insulin, which showed promise as a food supplement. However, it was confirmed that prickly pear fruit mainly had no significant impact on glucose or insulin. There seemed to be a different quality of evidence depending on the *Opuntia* spp. products. Studies using



fruit or cladode individually have a high risk of bias, whereas studies using collective *Opuntia* spp. products were less vulnerable to prejudice.

Various actions were suggested for positive effects, focusing on dualistic, glucose-dependent, and autonomous effects, but mechanisms also need further explanation. The authors concluded that the advice of using *Opuntia* fruit products as an alternative or complementary therapy to minimize the risk or to treat T2DM lacks evidence. However, the cladode makes promises for future glucose-reducing effects requiring more analysis.

## 2.2 Cancer

The series of conditions caused by uncontrolled malignant cell proliferation is recognized for cancer. The burden of neoplastic disease is a significant global health issue that kills thousands of people (Omara et al., 2020). Conventional cancer treatments have various side effects due to lack of precision and are confined in rural areas (Nguyen et al., 2019). Moreover, cancer cells' robust resistance to antineoplastic and cytotoxic drugs has raised a new problem, with unsatisfactory findings and resistance to antineoplastic agents (Luqmani, 2005; Singh & Settleman, 2010).

Many chemotherapeutic drugs for cancer treatment have been isolated from plants or their synthetic derivatives (Abid et al., 2020). More than 5000 phytochemicals from over 3000 plant organisms, including carotenoid, phenolic, terpenoid, glucosinolate, and alkaloid, have been identified as the main elements in cancer therapy (Graham et al., 2000; Solowey et al., 2014). As per global reports, *Opuntia* spp. is one of the most studied plants for its anticancer activity (Omara et al., 2020). Most studies showed that *Opuntia* spp., as nopal (cladodes or stems), fruits, or fruit juice could afford a remarkable anticancer approach (Del Socorro Santos Díaz et al., 2017).

Sreekanth et al. (2007) stated that the human chronic myeloid cell line K562 was inhibited by betanin, isolated from the fruits of *O. ficus indica*, through the intrinsic apoptotic pathway. Furthermore, in several cancer lines, Chavez-Santoscoy et al. (2009) tested the cytotoxic effect of filtered juices on different forms of *Opuntia* prickly pears with normal fibroblasts as control cells. The most affected cells were the PC3 and the Caco2 colon lines, while the development of the hepatic HepG2 and mammary MCF-7 was reduced to a lesser extent. *O. rastrera rastrero* was the utmost cytotoxic species with the best quality and ability of antioxidants among the different species evaluated.

Hahm et al. (2010) reported that the partitioned water fractions of *O. humifusa* stems and fruits increased ROS production in U87MG glioblastoma cells and suppressed their growth. Also, Serra et al. (2013) showed that natural extracts from juice residues (peels and seeds) of various *Opuntia* spp. were more effective to induce a cell-cycle HT-29 colon cancer cell lines than polyphenol-rich juice concentrates. Both products were not cytotoxic to Caco2. Interestingly, the effect was concomitant with an increase in ROS in the cells, which indicates that ROS producing cell death is likely caused by the extract's prooxidant effects. Feugang et al. (2010)

also reported this prooxidant effect in ovarian cancer cells compared with immortalized or normal cells.

Li et al. (2014) reported the anti-tumor effect of cactus polysaccharides of *O. dillenii* on the lung's squamous cell carcinoma. Using two models of human colon cancer cell lines, i.e., Caco2 and HT-29, which represent both apoptosis susceptible and apoptosis-resistant cell lines, Antunes-Ricardo et al. (2014) assessed the cytotoxic effects of purified isorhamnetin glycosides or flour extracts of *ficus indica* cladode while normal fibroblasts were used as controls. The authors stated that cladode flour extracts and distilled isorhamnetin glycosides had a cytotoxic effect on HT-29 cells rather than Caco2 or fibroblasts in a glycosylation way, promoting caspase-apoptotic pathways. Naselli et al. (2014) reported that aqueous extract of *O. ficus indica* fruit and its betalain pigment indicaxanthin showed a dose-dependent apoptotic effect proliferating the human colon cancer cell line Caco2 with no impact on differentiated cells. In particular, indicaxanthin demethylate and subsequently activate the tumor suppressor gene p16INK4a.

Antineoplastic activity against the HepG2 cell line was reported for the ethyl acetate fraction of the *O. stricta* flower extract (Prabhakaran et al., 2017). Antunes-Ricardo et al. (2019) reported that isorhamnetin glycoside of *O. ficus indica* induced apoptosis in human colon cancer cells (HT-29) via mitochondrial impairment. Abid et al. (2020) reported that HeLa cells treatment with *O. Monocantha* extracts evoked apoptosis, constrains proliferation, and improved the anti-oxidative index in post-treated cells.

Indicaxanthin is a dietary betalain bioactive molecule isolated from the *O. ficus indica* (Butera et al., 2002). Using collective reverse pharmacophore mapping, reverse docking, and text-based database search, Tutone et al. (2018) recognized various potential targets for indicaxanthin like glutamate carboxypeptidase ii, phosphoserine phosphatase, leukotriene-a4 hydrolase, phosphodiesterase 4d, inositol trisphosphate 3-kinase, and kainate receptor. These targets are expressed or overexpressed in cancer cells of many organs like the breast, thyroid, and prostate. In this respect, Allegra et al. (2018) demonstrated that indicaxanthin from *O. ficus indica* inhibited proliferation of A375 human melanoma cell line in vitro and impaired cutaneous melanoma development in vivo in mice model. Moreover, in a recent study of Allegra et al. (2020) in HeLa cells, the oxidative stress-dependent p53/p22 axis of indicaxanthin synergistically enhanced cisplatin-induced apoptosis.

A significant advantageous property for any anticancer agent is the selective cytotoxicity via cellular growth rate slowdown in cancer cells only without impacting normal cells. Thus, to conclude the potentially beneficial effect of phytocompounds, relevant controls are needed, namely cells of the same type with a similar genetic background. The compounds should be more cytotoxic to cancer cells than normal ones if they are qualified to protect against cancer. In this regard, Kim et al. (2015) showed that *O. humifusa* cladodes extract induced apoptotic events in human colon SW-480 and MCF-7 cells. The same team has reported an identical effect on HeLa cervical carcinoma cells, although normal fibroblasts have not been affected (Hahm et al., 2015). Besides, Keller et al. (2015) documented a protecting effect of numerous *Opuntia* cladode flour on the cytotoxic effect of 4-hydroxynonenal, a

dietary lipid oxidation agent that is likely involved in the red meat promoting impacts on colon cancer. Also, Lefsih et al. (2018) indicated that both deproteinated water-soluble pectin and heat-modified deproteinated water-soluble pectin from *O. ficus indica* selectively suppressing human neuroblastoma LAN5 cancer cells growth, without any negative impact on normal fibroblast cells.

The in vivo studies are very essential to approve those effects shown in vitro. Zou et al. (2005) demonstrated that the aqueous extract of *Opuntia* repressed nude mice's tumor growth to a level similar to the standard chemopreventive agent, the synthetic retinoid N-(4-hydroxyphenyl) retinamide. *O. humifusa* protecting effect was also described on HeLa cells xenografts (Hahm et al., 2015). The *O. ficus indica* cladode extracts protective effect on some mycotoxins (i.e., aflatoxin B1 and zearalenone) induced oxidative stress, and genotoxicity has been reported (Brahmi et al., 2011; Zorgui et al., 2009; Zourgui et al., 2008). *Opuntia* extracts were administered most of the time intraperitoneally. Thus, further research are warranted to validate the beneficial effects of *Opuntia* spp. by physiologically measuring certain compounds or by oral route for example that takes into account their digestibility and bioavailability. In this respect, the lyophilized powder of *O. humifusa* fruit was reported to protect against two separate skin carcinogenesis animal models and reduce skin lipid peroxidation and inflammation when given in the pelleted diet (Lee et al., 2012, 2013).

Notably, some reports revealed the non-cytotoxic effect of some parts of *Opuntia* spp. despite the other parts of the same species' cytotoxic effects. For instance, Izuegbuna et al. (2019) reported that while the *O. stricta* essential oil contains some cytotoxic compounds, their cladodes showed non-cytotoxic effects against U937 cell lines. The authors suggested that the compounds may not be present in adequate amounts. The low levels of phytochemicals produced in this study may also explain this. This non-cytotoxic effect was likewise detected by Gebresamuel and Gebre-Mariam (2011) in their experiment on *O. ficus indica* and *O. stricta* cladodes. Recently, Harrabi et al. verified the *O. stricta* cladodes extracts cytoprotective effect on HepG2 cells (Harrabi et al., 2017). Besides the phytochemicals level, seasonal variation, soil, and other variables can affect the plant's level of these compounds (Saravanakumar et al., 2015). While the literature cited higher summer yields, the cladodes were harvested in winter in this report. Similarly, in a study of the cytotoxic effect of various *Opuntia* spp. as *O. stricta*, the seasonal variations influenced the herb's phytochemicals and nutrients distribution. None of the cladodes showed cytotoxicity to the cell lines used Alves et al. (2016).

### 2.3 Cardiovascular Diseases

Cardiovascular disease (CVD) is considered the world's leading cause of disability and premature death (Song et al., 2020). CVD affected an estimated 422.7 million individuals in 2015 and an estimated 17.9 million deaths in 2015 globally, representing 31% of total global deaths, based on Global Burden of Diseases, Accidents,

and Risk Factor Report 2015 (Mozaffarian, 2017; Roth et al., 2017). By 2030, it is expected that about 23, six million people die of CVD every year (WHO, 2019).

CVD comprises coronary artery diseases like myocardial infarction and angina (Mendis et al., 2011). Other CVDs include stroke, abnormal heart rhythms, heart failure, rheumatic heart disease, hypertensive heart disease, cardiomyopathy, peripheral artery disease, valvular heart disease, congenital heart disease, thromboembolic disease, and venous thrombosis (Naghavi et al., 2015).

There is a growing interest in the *Opuntia* spp. nutritional benefit to inhibit the CVD development. Lipid-reduction and antioxidant features of the different *Opuntia* spp. may help their effectiveness in preventing or slowing down the development of atherosclerotic lesions and consequent CVD. *Opuntia*'s anti-atherogenic effects derive from its potent antioxidant content (phenolics), which may decrease the lipid peroxidation, a significant risk factor in atherosclerosis and dietary fiber and protein with lowering lipid content in humans and animals (Osuna-Martínez et al., 2014).

Regulation of cholesterol is essential for cardiovascular disease prevention. Padilla-Camberos et al. (2015) investigated the *O. ficus indica* aqueous extract hypocholesterolemic activity in mice who received triton. The results revealed that the extract might prevent hypercholesterolemia by inhibiting pancreatic lipase, in part because of its phenolic compounds. Wang et al. (2015) investigated the *O. dillenii* polysaccharide beneficial effects on atherosclerotic rat models fed a high-calcium and high-fat diet. These results showed that these polysaccharides could significantly enhance the thoracic aorta vasorelaxation in the atherosclerotic rats and has substantial anti-atherosclerotic activity; hindering the apolipoprotein B and diglyceride acyltransferase expression and consequently reducing the levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) serving as one of the molecular mechanisms of its antiatherosclerosis effect.

Keller et al. (2015) investigated the protective effect of cladode powder of *Opuntia* on LDL oxidation, a significant risk factor for developing atherosclerosis induced by vascular endothelial cells. The powders significantly inhibited the oxidation of LDL resulted from cultivation with murine endothelial cells. Also, in vivo studies on apoE-KO mice, which naturally progress atherosclerotic lesions in basal diet circumstances, Garoby-Salom et al. (2016) showed the *O. ficus indica* or *O. streptacantha* powdered cladodes addition to the diet at the level of 10 mg/kg bwt for 15 weeks considerably declined the development of the atherosclerotic lesion. However, Osorio-Esquivel et al. (2012) reported that *O. ficus indica* or *O. streptacantha* cladodes intake did not decrease the plasma's cholesterol level. This difference may come from a diet or *Opuntia* components (basal or cholesterol-enriched) (cladodes vs. seeds) (Del Socorro Santos Díaz et al., 2017).

Giglio et al. (2020) reported in a four-week intervention study that pasta's weekly use with *O. ficus indica* extract supplements enhanced the metabolic parameters and decreased atherogenic, dense, low-density lipoproteins in patients with metabolic risk factors. Also, Gouws et al. (2020) performed a systematic review that studied the modulatory role of *Opuntia* spp. parts (cladode or prickly pear) consumption on the risk factors for CVD development, especially TC, TG, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). In most

studies, prickly pear consumption was correlated with substantial decreases in TC while LDL C was reduced. Distinctly, the effect of cladode intake on lipids was minor. One study recorded a considerable rise in plasma HDL-C in a subset of individuals (>45 years of age) following ingesting of a patented cladode powder product. It is possible that the differences between cladode and pear can cause variations in total impact, for example, the composition of the fibre. Hence, the identity of the selected components of *Opuntia* spp. needs to be carefully recorded in future studies.

A systematic analysis was performed by Cheok et al. (2020) to determine the physiological effects in animal models and human studies of cacti on endothelial and vascular function. Animal studies have shown that vasodilation and serum nitric oxide can increase, and blood pressure and vascular rigidity may be decreased. A few human studies have shown reduced heart rate as well as an improvement in cardiac variability. Although the results seem to suggest enhanced vascular health, rigorous, randomized human intervention trials are lacking to establish underlying mechanisms, optimum doses, and long-lasting effects of cacti intakes.

The *Opuntia* spp. lipid-lowering activity could be positively related to their fiber content rather than their polyphenol content's antioxidant activity. Antioxidants prohibit lipid peroxidation but typically have no impact on lipid plasma profiles, except for grape phenolics (for example, resveratrol), which reduce plasma triglyceride levels and affect very-low-density lipoprotein cholesterol metabolism (VLDL) (Zern et al., 2005). Hence, in the case of *Opuntia*, the lipid decreasing properties may be rather a consequence of their dietary fiber content, as supported by Wolfram et al. (2002) study. The authors suggested that the *O. robusta* prickly pears lower the TC level in non-diabetic patients with hyperlipemia. Zern et al. (2005) correlated the *Opuntia* prickly pear hypolipidemic effect to their pectin content. The pectin mechanisms may lead to liver cholesterol changes without disturbing the absorption of cholesterol (Garcia-Diez et al., 1996; Gunness & Gidley, 2010). As well, the intense hypolipidemic and antioxidant activity of *O. ficus indica* glycoprotein was demonstrated to protect mice treated with tritone WR-1339, a lipoprotein lipase inhibitor (Oh & Lim, 2006). *Opuntia* prickly pears consumption boost platelet function and hemostatic equilibrium so that the atherosclerotic risk is prevented (Zern et al., 2005).

## 2.4 Obesity

Obesity is a complex chronic disease that threatens to reverse the life expectancy gains achieved over the past two centuries (Hruby & Hu, 2015; Zylke & Bauchner, 2016). It impacts lower social and ethnic minority groups overwhelmingly and has become one of the most significant global health issues of the twenty-first century (Danielli et al., 2021). The rise in obesity and overweight has contributed to the growth in various dietary supplements with different efficacy considerations. The scientific community increasingly shows an interest in antiobesity agents derived

from natural products, and some of their active compounds have been clinically investigated. One of these supplements is *Opuntia*, which has a significant antiobesity effect (Onakpoya et al., 2015a).

Cárdenas Medellín et al. (1998) explored the *O. ficus indica* cladodes antiobesity effects in rats. They showed that rats fed 12% nopal had lesser weight gains than the 6% nopal or control diet-fed ones. The obese Zucker sugar rat model has also been used by Morán-Ramos et al. (2012) to assess the possible antiobesity and lipid reducing effects of *O. ficus indica* nopal. By feeding rats over 7 weeks with 4% nopal, the liver TG was decreased by about 50%, and other histological and biochemical markers improved. This all indicated an increase in liver steatosis by augmenting the VLDL synthesis and fatty acid oxidation, suppressing oxidative stress, and enhancing hepatic insulin signaling.

In a randomized, double-blind, placebo-controlled clinical study, Grube et al. (2013) assessed the effect of litramine, a natural fiber complex of *O. ficus indica*, consumption 12-week combined with moderate exercise and a hypocaloric diet on overweight and obese volunteers. A 5% weight loss from the volunteers' initial body weight was recorded relative to placebo. A considerably higher reduction in body fat composition, body mass index, and waist circumference was recorded. They noted that the complement of litramine fibres was well tolerated, and no adverse effects were recorded. These findings show that the complex natural fibre litramine can promote weight loss effectively.

Chong et al. (2014b) reviewed the studies evaluating the Litramine IQP-G-002AS, a fiber derived from *O. ficus indica*, safety, and efficiency to reduce dietary fat absorption to endorse weight loss. Published and unpublished evidence from placebo-controlled randomized experiments shows promising fecal fat excretion, weight loss promotion, and body weight maintenance. The safety evaluation has also shown minimal concern, as the assessed studies have demonstrated that litramine IQP-G-002AS consumption is well endured. Besides, for the litramine in dosages tested, no significant gastrointestinal side effects were identified.

Onakpoya et al. (2015b) have reviewed a meta-analysis of randomized clinical trials to assess the evidence for the effectiveness of *O. ficus indica* for weight reduction and lipid-lowering effect. The authors concluded that *O. ficus indica* consumption could substantially decrease body fat percentage, total cholesterol, and blood pressure.

In a 12 weeks experiment in mice fed high-fat diet (HFD) model of obesity, Rodríguez-Rodríguez et al. (2015) assessed the *O. ficus indica* extract metabolic effect. The results showed that mice fed HFD diet but fortified with *O. ficus indica* extract had less body weight gain and displayed considerably lower TC, HDL-C, and LDL-C relative to mice fed non-supplemented HFD. In this study, *O. ficus indica* extract prohibited the progress of metabolic disturbances linked with obesity due to an unbalanced diet.

In rats fed a high fat and sucrose diet, Sánchez-Tapia et al. (2017) evaluated the effectiveness of nopal, *O. ficus indica*, and intake to decrease the obesity metabolic consequences adjusting the gut microbiota and inhibiting metabolic endotoxemia. In this study, nopal significantly modified gut microbiota, increased intestinal

occludin-1, decreased metabolic endotoxemia, glucose intolerance, glucose insulinotropic peptide, metabolic inflexibility, and lipogenesis. These improvements have decreased adipose tissue and brain oxidative stress and hepatic steatosis, besides enhanced cognitive performance, along with an increase in *B. fragilis*. This study promotes nopal as a functional food and prebiotic for altering intestinal microbiota and reducing metabolic endotoxemia and other biological abnormalities linked to obesity.

Bounihi et al. (2017) assessed the effects of *O. ficus indica* vinegar oral dosing for 18 weeks on body weight gain, visceral adipose tissue weights, cardiac enzymes, and inflammatory biomarkers in HFD induced obese rats. The vinegar mitigated the HFD-associated increase in body weight and visceral adipose tissue mass, in addition to the rise in blood levels of fibrinogen, CRP, leptin, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), aspartate aminotransferase, creatine kinase-MB, and lactate dehydrogenase. Besides, vinegar has maintained myocardial architecture and decreased cardiac fibrosis. These results indicate that prickly pear vinegar can prevent the incidence of HFD obesity and cardiac problems associated with obesity. The protection may derive from this vinegar's potent anti-inflammatory and anti-adiposity properties.

In HFD-fed mice, Yang et al. (2019) confirmed that the *O. humifusa* stem water-soluble polysaccharides significantly counteracted the HFD induced disturbances of hormonal and lipid profiles and decreased mass of fat. Furthermore, water-soluble polysaccharides enhanced the mice intestinal health by augmenting serotonin-positive and ghrelin-releasing cells and improve immunity by increasing the CD4+ cells nitric oxide synthase expression. Also, in HFD-fed rats, Héliès-Toussaint et al. (2020) reported that *Opuntia* cladode powders inhibited adipogenesis through adjusting metabolic indicators and favoring fat excretion in faeces.

Various suggestions on the possible effects of *Opuntia* extract in terms of energy metabolism, gene regulation, and regulation of insulin and glucose pathways indicated that cactus pears could be successful in controlling obesity, provided in different ways in the diet. Uebelhack et al. (2014) and Chong et al. (2014a) showed that *Opuntia* fibres bind to dietary fats and reduce their absorption, consequently increasing their excretion, leading to lower energy consumption and loss of weight. Moreover, animal research findings have indicated that the consumption of *Opuntia* can cause bodyweight reductions through diuresis (Bisson et al., 2010), enhance lipid profile by intracellular radicals quenching (Halmi et al., 2013; Medellín et al., 1998; Oh & Lim, 2006) and reduce blood glucose *via* increasing basal insulin level (Butterweck et al., 2011; Ennouri et al., 2006). Randomized, non-randomized studies in humans show that *Opuntia* can attenuate postprandial hyperglycemia by improving peripheral tissue glucose absorption (Deldicque et al., 2013b; Van Proeyen et al., 2012). Other humans studies have also verified that the *Opuntia* ingestion causes apparent declines in TGs, TC, and LDL-C in dyslipidemic patients through up-regulating hepatic LDL receptors binding (Palumbo et al., 2003; Wolfram et al., 2002).

Studies have found that *Opuntia* extracts such as flavonoid kaempferol or isorhamnetin can prevent lipids accumulation or induce adipogenesis through the

downregulation of adipogenic related genes (Lee et al., 2009, 2015). Overall, *Opuntia* affects the sensitivity of insulin favorably by regulating adipocyte differentiation-related genes (Kang et al., 2013), attenuates hepatic steatosis (Morán-Ramos et al., 2012), as well as reduce metabolic and cognitive defects and gut microbiota dysbiosis (Avila-Nava et al., 2017).

## 2.5 Inflammatory Bowel Disease

Inflammatory bowel diseases (IBDs) are chronic lifelong conditions with an idiopathic, chronic hereditary path (Cosnes et al., 2011). IBDs are characterized by an extreme pro-inflammatory mediators release, intestinal barrier malfunction, and disturbed permeability, and excessive nuclear factor kappa B (NF- $\kappa$ B) cascade activation that can initiate colon cancer growth. The disease affects every part of the gastrointestinal system causing edema, bleeding, ulceration, and fluid and electrolytes loss. IBDs include various chronic inflammatory disorders, mostly Crohn's disease and ulcerative colitis (Molodecky et al., 2012). These diseases are considered significant health complications in developing countries and spread to the rest of the world due to their high occurrence and prevalence, resulting in severe socio-economic problems (Sergent et al., 2010).

Herbal medication is favored in treating a wide variety of gastrointestinal acute and chronic illnesses. Preclinical and clinical studies have shown that plants' use in chronic inflammatory disorders such as IBDs is promising (Akkol et al., 2020). In this regard, usage of an intestinal inflammation in vitro cell-based model, Nunes (2011) reported that *O. ficus indica* phenolic-rich extracts significantly attenuated intestinal barrier impairment probably because of the tremendous antioxidant effect of its bioactive isorhamnetin and the high ability to modify activation of NF- $\kappa$ B thus suggesting a promising role of *Opuntia* in IBD treatment.

Aboura et al. (2017) explored the influences of phenolic-rich infusions from *O. ficus indica* cladodes on ulcerative colitis caused by dextran sodium sulfate in Swiss mice. The infusions prohibited the intestinal barrier's permeability *via* tight junction proteins (Zo1, occludins) restoration and relieve severe inflammation accompanying colitis because of their polyphenols content. Moran-Ramos et al. (2017) investigated the effects of nopal cladode ingestion on microbial community configuration, intestinal physiology, adipose tissue, and serum biochemistry in obese rats caused by feeding an unbalanced diet. Nopal intake decreased intestinal inflammation, modified gut microbiota structure, augmented microbial diversity, increased cecal fermentation, and changed the serum metabolome. Babitha et al. (2019) reported that fresh fruit juice of *O. dillenii* Haw. attenuated acetic acid-induced ulcerative colitis in rats, probably *via* its anti-inflammatory and antioxidant activities due to phenolics, flavonoids, and betalains in the fruit juice.



## 2.6 *Alzheimer's Condition*

More than 50 million people are reportedly living with dementia worldwide, with the number of people concerned projected to rise to 66 million by 2030 (Prince et al., 2013). Alzheimer's disease (AD) is the leading cause of elderly persons' dementia and disability (Di Iulio et al., 2010; Verdaguer et al., 2020). The 2012 World Health Organization showed that over 35.6 million people worldwide had dementia, with about 60–70% of the population suffering from AD (Blennow et al., 2000). AD is a neurodegenerative disorder that leads to memory loss and cognitive decline (Cummings & Cole, 2002). Natural antioxidants plentiful in many medicinal plants have an outstanding free radical scavenging ability to combat AD and other oxidative stress-related neurological disorders (Abd-Elhakim et al., 2020; Behairy et al., 2020; Bui & Nguyen, 2017; Ciccone et al., 2020; Panda & Jhanji, 2020).

Allegra et al. (2015) have shown that orally administered indicaxanthin, derived from *O. ficus indica*, at dietary doses can cross the blood-brain barrier (BBB) and store in the rat-brain and, further, that the phytochemical's fascinating ability to modulate hippocampal neurons bioelectrical activity has for the first time emerged. These phytochemicals are present in various concentrations, but not in the stretch-pallidal complex, in the cortex, hippocampus, diencephalon, brainstem, or cerebellum. Its unique ability to cross the BBB may be because of its lipid membrane affinity, amphiphilicity, and high bioavailability (Turco Liveri et al., 2009). The selective indicaxanthin pigment accumulation in some brain sections and excluding others such as the striatopallidal complex may provide a more complex scenario. Anatomically, the difference may be due to the specific structural features of the basal ganglia subcortical region; the existence of numerous fiber bundles encapsulating the striatum and pallidum cannot, in particular, allow the accumulation of indicaxanthine just as smoothly as in other brain parts (Parent & Hazrati, 1995). The indicaxanthin chemical structure could also explain its lack in specific brain regions (Andres-Lacueva et al., 2005).

Gambino et al. (2018) demonstrated that after local injection of indicaxanthin, neurons' bioelectric activity from diverse brain regions was modified mostly with dose-related activities. An overall inhibitory effect indicates that indicaxanthine may have a potential new beneficial effect on cell excitability. These results may be a new base for investigating biological mechanisms by which indicaxanthin may modulate neuronal function by repeated complex cognitive brain processes and associated neurodegenerative conditions.

*O. ficus indica* peel, cladode, and fruit pulp extracts have been reported to exhibit considerable antioxidant effects in vitro and neuroprotective capacity in aluminum chloride associated AD features (El-Hawary et al., 2020). Notably, cladodes produce the highest phenolic content of the tested extracts and display the highest antioxidant, anti-inflammatory, and neuroprotective activity that could be triggered by the existence of many phenolics, which could function as acetylcholinesterase level and serotonin transporter inhibitors.

## 2.7 Parkinson's Disease

Neurodegenerative disorders are a major social challenge as they cause a wide variety of symptoms that inexorably worsen with age and have a significant decrease in life expectancy (Bayer, 2015). Parkinson's disease (PD) is a highly prevalent neurodegenerative proteinopathy and is considered the most prevalent movement disorder associated with age (Bertram & Tanzi, 2005). Studies that identify plant-based small molecules with promising neuroprotective activities, particularly in PD, have seen a rise in recent years (Caruana & Vassallo, 2015; Rigacci & Stefani, 2015).

Briffa et al. (2017) investigated whether extracts derived from *O. ficus indica* could relieve neurodegenerative events in the fly (*Drosophila melanogaster*) and yeast (*Saccharomyces cerevisiae*) models of PD. The findings suggested that *O. ficus indica* extract impedes with mutual mechanisms of PD associated neurodegeneration. This definition is supported by evidence that *O. ficus indica* extract, though powerfully hindering the A $\beta$ 42 and  $\alpha$ -syn fibrillogenesis, collects remodeled oligomeric collections that are less efficient disturbing the integrity of membrane lipid. These newly discovered features of *Opuntia* spp. opened up new possibilities for improving the treatment of amyloidogenic neurodegenerative disorders with therapeutic applications of different plant products.

## 2.8 Arthritis

Arthritis is a complex condition that not only causes physical weakness, but decreases the quality of life when bone and joint pain are gradually destroyed (Rudan et al., 2015). Present traditional anti-rheumatic drugs do not deliver full remission, do little to delay the disease's development, and have severe complications (Harirforoosh et al., 2013). Natural product use has been proven successful as non-steroidal anti-inflammatory medications, reduced toxicity, improved potency, and increased bioavailability to alleviate osteoarthritis symptoms (Roome et al., 2019).

In human chondrocyte culture, motivated with interleukin-1 $\beta$ , a proinflammatory cytokine, Panico et al. (2007) explored the chondroprotective and anti-inflammatory effect of *O. ficus indica* cladodes lyophilized extracts and hyaluronic acid (HA) on the release of main molecules produced in the chronic inflammatory events as glycosaminoglycans, nitric oxide, and prostaglandins. Additionally, the extracts' anti-oxidant activity was assessed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. In this analysis, all the extracts examined displayed an exciting profile of inactive substances. Some of them have been characterized by polyphenolic and polysaccharide species in particular in vitro findings verified that the *O. ficus indica* cladodes extract significantly counteracted the IL-1 $\beta$  detrimental impacts. Besides, the authors confirmed the *O. ficus indica* cladodes extracts protective against

cartilage impairment, which seems superior to that provoked by HA frequently used as visco-supplementation in the joint treatment disease.

Sharma et al. (2017) validated the *O. humifusa* use in Korean Oriental Medicine as an anti-inflammatory and an analgesic agent in rheumatoid arthritis cases. Additionally, their results proposed that the *O. humifusa* methanol extract had anti-inflammatory and anti-nociceptive effects through both the peripheral and central systems. Roome et al. (2019) studied the anti-arthritis activity of two chemical components derived from *O. dillenii* cladode named opuntioside-I and opuntiol and its silver and gold nanoparticles in rats model of complete Freund's adjuvant-produced arthritis. This study concluded that, due to their anti-inflammatory and immunomodulatory capacity, the disease progression has successfully been restored by the tested compounds and nano-formulations in the complete Freund's adjuvant-induced arthritic rat and can be considered for arthritis-targeted therapy to meet the most significant challenges of the disease.

## 2.9 Chronic Wounds

Wound healing is a complex, highly controlled procedure important to preserving the skin's barrier function. The cascade of events involving the cure of wounds can be disrupted with different disease processes, leading to chronic, non-healing wounds that cause severe pain and distress in the drainage of a considerable number of medicines (Han & Ceilley, 2017). It has been estimated that the total cost of chronic wounds is over US\$25bn a year, and annually there are 6.5 million patients affected due to increased prevalence of diabetes and other chronic conditions that impair wound healing (Sen et al., 2009).

Various traditional herbs and plant parts have been shown to efficiently treat skin injuries (Kamath et al., 2003; Leach, 2008; Roy et al., 2012). Of particular interest, nopal and other *Opuntia* spp. extracts have been used for many years in conventional medicine to treat burns, skin conditions, and injury, and the recent evidence of their effectiveness at molecular and cellular levels justifies their use in today's dermatological preparations (Ribeiro et al., 2015). Numerous latest indicated the *O. ficus-indica* cladode extracts wound-healing effects. Overall, these studies emphasized the *Opuntia* spp. capacity to hasten wound healing and their probable importance in managing skin complications in DM and other pathologies associated with a flawed wound healing.

Park and Chun (2001) have confirmed the substantial wound healing effects of *O. ficus indica* cladodes methanol extract. Trombetta et al. (2006) have also displayed that *O. ficus indica* cladodes lyophilized polysaccharide extracts positively impacted skin wound healing. The authors assumed that these polysaccharides' smooth structure and hygroscopic, rheological, and viscoelastic properties could be substantial for wound-healing promoters.

In an excision wound model in rats, Ammar et al. (2015) confirmed that using the *O. ficus indica* flowers methanol and mucilaginous extracts for thirteen days resulted

in remarkable cutaneous repair represented by accelerated wound contraction and remodeling phases, suggesting its potential role in wound healing. Using benzopyrene or TNF- $\alpha$ -stimulated keratinocytes, Nakahara et al. (2015) have shown that *O. ficus indica* cladode extracts can protect the epidermal barrier and function of keratinocytes by upregulating filaggrin and loricrin expression, the two proteins in distinguished keratinocytes and corneocytes. The protection effect of the extract is characterized by inhibition by the inflammatory agents of the ROS development. This could arise from the aryl hydrocarbon receptor activation, which activates the Nrf2 transcript factor and consequently the NAD(P)H: quinone oxidoreductase 1 antioxidant.

Petruk et al. (2017) demonstrate that the water extract from *Opuntia* has strong antioxidant properties that can counteract the adverse effects of UVA radiation on the human keratinocyte and protect cells from this widespread and pernicious source of stress. Eucomic and piscid acids must be the anti-stress effect of cladode extracts since the only elements that preserve the extract's antioxidant function are these phenolic compounds. For both acids, the powerful antioxidant effect is demonstrated in its structure, promoting hydrogen atoms' transfer to free radicals. Thus, the antioxidant extract of aqueous *Opuntia* can be obtained by simply pressing cladodes to prevent the use of expensive products and chemical solvents responsible for environmental degradation and residual persistence that are harmful to human health. This could result in *Opuntia* extract being a bioactive, healthy, cost-effective, and cost-effective extract used for pharmaceutical or cosmetic applications for skin health/protection applications. Also, Bassino et al. (2019) assessed the *O. ficus indica* effects on adult keratinocytes' function (HaCaT) under basal situation or in case of mechanical damage like wounded cells. *Opuntia* contrarily improved the metabolism and the HaCaT migratory properties in normal conditions and upon mechanical damage. Besides, it modulated the HaCaT reaction to oxidative stress.

The *O. ficus-indica* cladodes wound healing effects may comprise high molecular weight polysaccharide components like a linear galactan polymer and a highly branched xyloarabinan to low molecular weight elements like D-mannitol, lactic acid, eucomic, piscidic, and 2-hydroxy-4-(4'-hydroxyphenyl)- butanoic acids. These extracts could hasten cell reinforcement on a scratched keratinocytes monolayer, proposing that *O. ficus indica* components display great anti-inflammatory and high wound-healing effects (Deters et al., 2012). Likewise, Deters et al. (2012) reported that *O. ficus indica* polysaccharides stimulate fibroblasts and keratinocytes proliferation. Among the protective elements in these extracts, isorhamnetin glycoside components such as diglycoside isorhamnetine-glucosyl-rhamnoside can inhibit COX-2, the development of TNF- $\alpha$ , IL-6, and the generation of lipopolysaccharide-evoked nitric oxide (Antunes-Ricardo et al., 2015). Besides, *O. humifusa* cladode extracts can regulate HA production by increasing synthase expression in keratinocytes exposed to UV-B therapy. In comparison, treatment with *O. humifusa* cladode extracts can decrease the hyaluronidase's increased UV-B expression. It is important to note that HA was protected by the same protective action on SKH-1 hairless moustaches exposed to UV-B, suggesting the excellent skincare potential of *O. humifusa* cladode extracts (Park et al., 2017).

## 2.10 Alcoholism

Excessive consumption of alcohol induces a variety of illnesses and is therefore of great social concern. Global alcohol use over the past decades has increased by several times (Mehta, 2016). In other words, action and retroactive steps are urgently needed. Modern pharmacological solutions induce absolute self-restraint of alcohol and avoid rebound but have many adverse consequences (Hasin et al., 2007). There are records of a variety of medicinal plants and purely natural compounds having protective and therapeutic effects on alcoholism and alcohol dependence, but the key elements, effectiveness, and mechanisms of action are still unclear (Singh et al., 2019; Tomczyk et al., 2012).

Few studies have assessed the *Opuntia* spp. benefits in treating alcohol hangover symptoms in humans. For example, Wiese et al. (2004) carried out a double-blind cross-checking trial of 64 adult volunteers with placebo checking. Volunteers were given two *O. ficus indica* extract capsules and equivalent placebo 5 h before alcohol use in this randomized study. Subjects ingested alcohol up to 1.75 g/kg wt for 4 h. Strictness and general health were tested for a hangover, and the next morning, blood and urine samples were taken. The results revealed that the *O. ficus indica* consumption reduced alcohol hangover signs like dry mouth, anorexia, and nausea. The CRP levels have been closely correlated with the hangover's severity as the subjects with morning CRP levels greater than 1.0 mg/L; the average sign index was higher. The hangover severity could be partly because of inflammation and lipid metabolism homeostasis disturbances caused by alcohol drink impurities and alcohol metabolism by-products (Chauhan & Kulkarni, 1991). Thus, the *O. ficus indica* extract has a moderate effect in reducing hangover symptoms, seemingly hindering inflammatory mediators' production. However, the systematic search conducted by Pittler et al. (2005) does not indicate that the supplementation of the food supply by *O. ficus indica* extract is useful for the treatment or prevention of alcohol hangovers. Thus, future research should analyze the changes in an alcohol-induced hangover and the plant-based products modulatory roles in such changes.

## 2.11 Mood Disorders

Depression is a debilitating illness that can significantly interfere with the quality of life of a person. In clinical practice, depression is the second most common chronic illness (Whooley & Simon, 2000) and by 2020 will become the world's second most common source of early death or disability (World Health Organization, 1999). While several efficient antidepressants currently exist, approximately one-third of all subjects treated are often insufficient in therapy's existing armamentarium. Therefore, it is essential to develop new and more efficient antidepressants from conventional plants whose psychotherapeutic potential was tested in a broad range of animal models (Zhang, 2004). In this regard, Park et al. (2010) demonstrated that

the two flavonoids (quercitrin and kaempferol) isolated from the *O. ficus indica* had been reported to exhibit antidepressant activity at the oral dose of 50 mg/kg, using the forced swim test.

### 3 Safety Aspects of *Opuntia* spp.

While some health claim herbal medicines are reasonably healthy because they are “natural,” few supportive evidence supports such a hypothesis. As herbal blends can contain specific contaminants at any stage of the process, adverse consequences may also be correlated with plant components and human (intrinsic) and non-plant contaminant factors (extrinsic)(Gagnier et al., 2006).

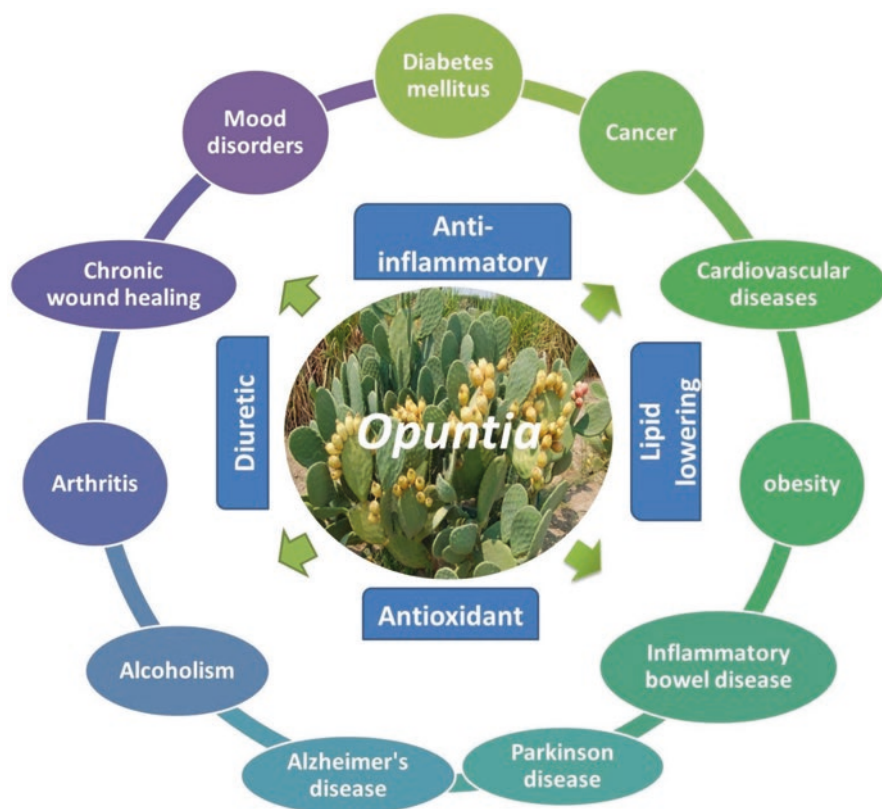
The existence of opportunistic pathogens/microorganisms or microbial toxins can even influence herbal compounds’ active ingredient content and cause adverse health effects. Plant-derived herbal interventions should also be cautiously handled or as potentially harmful chemicals unless otherwise shown (Aruwa et al., 2018).

Little information is available on *Opuntia* spp. toxicology and adverse side effects of its compounds or extracts (Aruwa et al., 2018). Despite the well tolerance of oral consumption of *O. ficus indica*. However, it has been recorded in traditional folk medicine books and case reports that it may cause nausea, mild diarrhea, headache, increased stool volume, increased headache, abdominal fullness, and low colonic obstruction (Kleiner et al., 2002). Moreover, extreme sensitivity to any *Opuntia* variable may be seen because individual pharmacokinetics differs widely. Dermatitis was a common adverse reaction to prickly pears (Pawar et al., 2017).

Recently, Han et al. (2019) evaluated the *O. ficus indica* genotoxicity and repeated oral toxicity. This study revealed that *O. ficus indica* administration (0, 500, 1000, and 2000 mg/kg/day orally for one week) did not change the normal rat behavior, body weight gain, and water and food intake. Moreover, during the ophthalmology test, no adverse effects were detected. The hematological and serum biochemical values and parameters for urinalysis and organ weights were all close to standard controls. Furthermore, according to the Ames test results, no mutagenic activity from the *O. ficus indica* was found in *E. coli* or *Salmonella typhimurium* with or without S9 activation. The *O. ficus indica* did not considerably change the Chinese hamster lung cells’ structural aberrations in the absence or presence of S9 activation. The *O. ficus indica* oral dosing also caused no substantial increase in the micronucleated polychromatic erythrocytes number in the mean ratio of polychromatic to total erythrocytes. Thus, *O. ficus indica* could be considered useful and healthy functional food or herbal medicines.

## 4 Conclusion and Health Perspectives

In recent years, a growing majority of studies have expanded awareness of the beneficial effects of nutraceutical products or dietary phytochemicals in treating chronic diseases. *Opuntia* spp. contain a vast collection of non-phenolic and phenolic components, which singly or synergistically exert significant biological effects against many chronic diseases like DM, Cancer, CVD, obesity, IBDs, AD, PD, arthritis, chronic wound healing, alcoholism, and mood disorders (Fig. 20.2). Notably, most safety studies support *Opuntia* spp. products' high safety, which is very advantageous for consumers and is affordable and co-effective.



**Fig. 20.2** Main biological activities and their roles in the treatment of chronic diseases

## 5 Recommendations

From the collective studies, despite the health-promoting effects of *Opuntia* spp., further knowledge about known and novel *Opuntia* compounds in their effective concentrations are still required. More sophisticated methods for the structural clarification of possible new and unidentified *Opuntia* compounds that may have new applications in other chronic diseases like autism spectrum disorders and epilepsy are also needed. More oriented research is also necessary to add toxicological knowledge for *Opuntia* extracts/herbal mixtures and their action mechanisms to protect human health.

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# Chapter 21

## Traceability of *Opuntia* spp.



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**Abstract** Traceability has established itself as an essential tool of the agri-food business to improve consumers' safety and confidence and support regulatory authorities in food control and fraud detection. Indeed, traceability has extensively demonstrated to profitably unmask frauds in terms of adulteration, species or even cultivar substitution, and not least, product provenance, by combining a variety of advanced analytical techniques with chemometrics. In this chapter, the state-of-the-art of *Opuntia* spp. traceability is discussed with particular emphasis to the species *Opuntia ficus-indica* (L.) Miller and its cultivars, originating from the Mediterranean area. At an earlier stage, studies chemically characterized fruits of the prickly pear cactus and its derived products (i.e., fruit juice and seed oil), already pointing out peculiar compositional profiles in dependence of the geographical provenance or even cultivar. However, only recently, the screening of minerals, volatile and phenolic compounds, was combined with the multivariate statistical analysis in an attempt to purposely trace the geographical origin of *O. ficus-indica*, as well as the cultivar it belongs. Overall, the traceability platforms designed so far may play a vital role in the product quality and safety assurance system. They revealed to preserve the authenticity of products from *O. ficus-indica* successfully, thus, protecting consumers against mislabeling and false information.

**Keywords** Chemical characterization · Authenticity · Geographical origin · Adulteration · Multivariate statistics · Chemometric

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## Abbreviations

AAS	Atomic absorption spectrometry
ANN	Artificial neural network
ANOVA	Analysis of variance
CDA	Canonical discriminant analysis
FES	Flame emission spectrophotometer
GAE	Gallic acid equivalents
GC-FID	Gas chromatography coupled to flame ionization detector
GC-MS	Gas chromatography coupled to mass spectrometry
HCA	Hierarchical cluster analysis
HPLC	High performance liquid chromatography
HSD	Honestly significant difference
HS-SPME/GC-MS	Headspace solid-phase microextraction coupled to GC-MS
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
INDE	Indicaxanthin equivalents
LC-MS	Liquid chromatography coupled to mass spectrometry
LDA	Linear discriminant analysis
MANOVA	Multivariate analysis of variance
MIR	Mid-infrared spectroscopy
MLR	Multiple linear regression
NGS	Next-generation sequencing
NIR	Near-infrared spectroscopy
NMR	Nuclear magnetic resonance
PC	Principal components
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCR	Principal component regression
PDA	Photo diode array detector
PDO	Protected designation of origin
PLS	Partial least squares
PLSDA	Partial least squares discriminant analysis
RMSEC	Root mean squared error in calibration
RMSECV	Root mean squared error in the internal cross-validation
RMSEP	Root mean squared error in the external validation
SIMCA	Soft independent modeling of class analogies
TAP	Traditional agri-food product
UHPLC-MS <sup>n</sup>	Ultra HPLC coupled to linear ion trap mass spectrometry
UNEQ	Unequal class-modeling

## 1 Traceability from a Food Perspective

With the advent of globalization in the food trade, keeping safety and quality along the supply chain has become a significant challenge. Several worldwide scandals and incidents (e.g., dioxin in chicken feed, bovine spongiform encephalopathy, genetically modified crops in food products, illicit use of quality logos, etc.) have threatened the credibility of the food industry and goods that come to the consumers' table as well (Aung & Chang, 2014). Consequently, on the one hand, consumers have shown increasing concern about the safety/quality of the food they eat, being aware that the information available in labeling does not always translate into greater confidence. The various actors of the food sector, on the other, have sought the higher quality of the raw materials introduced into the food chain, required certification and accreditation of products, and demanded safety/quality management systems, with the final aim of preventing fraud and unfair market competition (Aung & Chang, 2014)

In this context, traceability has become a priority for ensuring the safety/quality requirements of food, increasing the transparency of the supply chain, facilitating the internationalization of many products, and the food industry's overall growth (Regattieri et al., 2007). The term "traceability" is nowadays intended with a broad meaning and used more than ever by organizations, legislations, and the production industry, being the food segment not excluded. According to ISO 9000 (2005) quality standards, traceability is defined as "the ability to trace the history, application or location of any item or its characteristics through recorded identification data". The ISO guidelines further specify that traceability may refer to the origin of materials and parts, the processing history, and the product's distribution and location after delivery. The European Union (EU) regulation 178/2002 (European Commission, 2002) narrows the definition to the food industry, describing it as "the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing, and distribution". The Codex Alimentarius Commission (CAC, 2005) defines traceability as "the ability to follow the movement of a food through specified stage(s) of production, processing, and distribution".

In light of these definitions, many applications of traceability are to be expected. For example, traceability may monitor food products' location along the chain and facilitate their recall when safety and quality standards have been breached (Opara, 2003; Regattieri et al., 2007; Galvez et al., 2018). Alternatively, traceability may assure food safety/quality by verifying the compliance with its label description. This implies the determination of the geographical origin, the production method, and, not least, the plant or animal species present in a foodstuff (Españeira & Santaclara, 2016).

Strategies employed in the so-called "food authentication" have typically relied on the synergistic use of advanced analytical techniques and chemometrics mainly because of the high sophistication level of fraudulent procedures (Bertacchini et al., 2013). The most common instrumental techniques include, but are not limited to,

liquid and gas chromatography coupled to mass spectrometry (LC-MS and GC-MS), inductively coupled plasma mass spectrometry (ICP-MS), spectroscopic techniques (i.e., vibrational spectroscopy, fluorescence spectroscopy, nuclear magnetic resonance [NMR], mid and near-infrared spectroscopy [MIR and NIR]), and DNA-based techniques, such as polymerase chain reaction (PCR), and next-generation sequencing (NGS) (Peres et al., 2007; Espiñeira & Santaclara, 2016; Montet & Ray, 2017; El Sheikha et al., 2018; Lo & Shaw, 2018; Wadood et al., 2020). Each technique has got its pros and cons. GC and LC-based techniques are highly sensitive, robust, and reproducible as well. They have been long employed mainly in the geographical and botanical authentication of agro-products, as they can detect a wide array of metabolites and volatiles exploitable as traceability markers (Wadood et al., 2020). Nevertheless, such approaches are time-consuming, expensive, and require laborious sample preparation procedures (Kamal & Karoui, 2015). ICP-MS is even more sophisticated, sensitive, and reliable for traceability purposes. Multi-element and multi-stable isotope analysis has been widely used as a tool for tracing plant and animal foods' provenance, as their isotopic and element profiles are strictly related to the growth environment they come from (Suzuki & Nakashita, 2013; Potortì et al., 2013, 2020). Also, ICP-MS has been demonstrated to be very useful in discriminating the botanical origin and the cultivar and plant products (Di Bella et al., 2016; Albergamo et al., 2017; Potortì et al., 2017). However, the requirement of a costly and complex instrumental system and highly trained operators constitutes a non-indifferent drawback of such a technique (Drivelos & Georgiou, 2012; Wadood et al., 2020). The accessibility to thousands of DNA markers and diverse sequencing methods make undoubtedly DNA-based approaches relevant to address many authenticity issues, including the discrimination of species and variety, geographical origin, mode of production, and the identification of genetically modified species, and uncontrolled admixtures both in plant and animal products (Voorhuijzen et al., 2012; Martins-Lopes et al., 2013; Zhao et al., 2017). Nevertheless, many shortcomings encountered during the experimental procedure (e.g., DNA fragmentation and cross-reaction of DNA from similar species present in the same food sample) could significantly alter the outcome (Lo & Shaw, 2018; Wadood et al., 2020). Spectroscopy provides signal profiles particularly complex and, thus, their assessment and interpretation are usually not straightforward. Also, such techniques often suffer from low sensitivity and interferences from water or air. Despite that, they are rapid, cost-effective, environment-friendly, and involve minimal or no sample preparation (Wadood et al., 2020).

Mathematically and statistically modeling the vast volumes of data obtained from the variety of analytical platforms, chemometrics removes bias and extracts only the meaningful information from the dataset, thus, improving the interpretation and the presentation of results (Badia-Melis et al., 2015). The benefits of tools based on multivariate statistics, over the univariate or bivariate approaches, show a good updating capacity and can be easily converted into a set of specifications that may help develop decision rules on the authenticity of food (Vandeginste, 2013). However, getting significant results requires meaningful data and the rational use of the multivariate method, and the understanding of the purpose of the analysis.

Based on the food authentication task, three categories of multivariate approaches can be outlined: (1) exploration, (2) calibration (necessary to unravel quantification issues), and (3) classification (necessary for adulteration and/or fraud detection) (Biancolillo et al., 2020). Exploratory tools, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), are usually conducted before food authentication study to assess, for example, the suitability of the analytical technique, such as ICP-MS, to differentiate food products with diverse geographical origin from their element profile (Bua et al., 2017; Mottese et al. 2018a, b). Exploratory methods enable data reduction with a minimum loss of original information, reveal hidden/underlying sample structures, and exploit plots or graphs to pinpoint similarities, differences, clusters, and/or correlations among samples and/or variables. These techniques are notoriously described as “unsupervised”, as they do not require any input (i.e., prior knowledge of sample group or labeling) other than the dataset itself (Bua et al., 2017; Mottese et al., 2018a; Potortì et al., 2018).

Regression techniques cover linear methods, such as multiple linear regression (MLR), principal component regression (PCR), partial least squares (PLS), and non-linear variants of the PLS algorithm, such as support vector machines and artificial neural networks (ANNs). These methods come in handy when the authentication issue implies the quantitative assessment of one or diverse adulterant constituents in food, for instance, the determination of fructose: glucose mixtures in “extended” honey (Toher et al., 2007), or lard content in chocolate products (Chen Man et al., 2005).

Different from the exploratory approaches, they are defined as “supervised”, as they require prior knowledge of samples, i.e., reference quantitative value(s) of food component(s), other than the experimental data. That is because a training or calibration stage is conducted using such information and becomes mandatory to establish a “regression model” able to predict the quantitative value of a food adulterant in unknown samples (Biancolillo et al., 2020).

Given the extreme relevance and the higher application rate they have for resolving real-world authentication issues, conventional and innovative classification approaches have been increasingly considered in the last decades (Bevilacqua et al., 2013). Indeed, they have been widely exploited to discriminate food with different provenance (Vitale et al., 2013; Firmani et al., 2019), varieties (Marini et al., 2004), technological properties (De Luca et al., 2016; Grassi et al., 2018), purity degree (Schiavone et al., 2020), and genotypes (i.e., transgenic/non-transgenic) (Xie et al., 2009). The classification relies basically on supervised discrimination and modeling methods.

Discriminant tools, such as linear discriminant analysis (LDA), partial least squares-discriminant analysis (PLS-DA), reveal differences among samples from distinct classes (Marini, 2010). However, they need to be prior calibrated by a training set consisting of already known samples belonging to distinct classes for building up a proper “classification model” useful for the prediction of the given information in a set of unknown samples (Albergamo et al., 2018; Mottese et al., 2020). Conversely, modeling procedures, such as soft independent modeling of class analogies (SIMCA) or unequal class-modeling (UNEQ), search for



similarities among samples belonging to the same class (Biancolillo et al., 2020). Specifically, these methods treat separately every sample category so that respective “class” or “category” models are constructed. Hence, these models can easily be applied (1) to predict cases from a single class, or (2) to predict new samples as members of one, none, or multiple classes (Marini, 2010).

In light of these premises, this chapter focuses on the efforts made so far to develop authentication models suitable to trace the geographical and botanical origin of *Opuntia* spp. Hence, particular attention will be paid to the potential of different analytical techniques combined with chemometrics tools in resolving some real issues related to ascertaining the authenticity of food products derived from such plant genus.

## 2 Tracing *Opuntia* spp.

### 2.1 General Background on *Opuntia* spp. and *Opuntia ficus-indica* (L.) Miller

*Opuntia* genus belongs to the Cactaceae family and includes over 180 species (Peña-Valdivia et al., 2008). It is endemic to America and has spread in diverse arid and semi-arid zones, characterized by droughty conditions, erratic rainfall, and poor soils subject to erosion. Nevertheless, due to a remarkable genetic variability and high ecological adaptivity, *Opuntia* is nowadays part of the natural landscape in North, Central, and South America, Mediterranean area, North, Central, and South Africa, Middle East, Australia, and India (Mondragon-Jacobo & Perez-Gonzalez, 2001; Stintzing & Carle, 2005). It is also cultivated in many countries globally, such as Italy, Spain, Mexico, Chile, Argentina, and California, by exploiting three main production systems, namely wild cactus communities, family orchards, and intensive plantations (Mondragon-Jacobo & Perez-Gonzalez, 2001). There is an increasing interest in opuntias, not only because they have become an endless source of products and functions, initially as wild plants and, later, as a crop for both subsistence and market-oriented agriculture, but also because they are likely to play a role in the success of sustainable agricultural systems in arid and semi-arid zones, where farmers must look to those few species that can profitably survive and produce (Mondragon-Jacobo & Perez-Gonzalez, 2001).

Among the various species, *Opuntia ficus-indica* (L.) Miller, commonly known as the prickly pear cactus, is native to Mexico and has subsequently propagated in Latin Americas, South Africa, and the Mediterranean basin (Hassan et al., 2011). This cactus is worldwide recognized as the opuntia and cactus species with the highest economic and social impact and scientific relevance as well, due to the abundant and profitable production of fleshy fruits (called “prickly pears”) and fleshy flattened stems (known as “cladodes”). The prickly pear consists of a thorny pericarp with several small prickles enveloping a luscious and sweet pulp purple,

yellow or white, and intermixed with a consistent number of hard seeds. It has a high commercial value both for the intense flavor and the excellent nutritional and pharmacological properties, especially in the presence of phenolics, betalain pigments, vitamins, and minerals (Piga, 2004). Hence, prickly pears are used for the manufacture of food products (e.g., juices, alcoholic beverages, jams, oil extracted from the seeds, and natural sweeteners) and cosmetics including creams, soaps, body lotions, and shampoos (Kaur et al., 2012).

On the other hand, the cladodes are modified stems that replace the leaves in their photosynthetic function. They are succulent and thorny organs with ovoid or elongated form, and they are rich in fiber, hydrocolloids, phenolics, carotenoids, minerals, and vitamin C (Sáenz et al., 2004; Rocchetti et al., 2018). The cladodes are of lower use for human consumption than fruits. However, the tender stems, known as “nopalitos”, are served especially in Mexico as juice, green salad, and soup mixed with other vegetables. Also, cladodes represent a useful source of animal feed and fodder, and they are widely employed in the pharmaceutical and cosmetic fields as starting points to produce dietary supplements and body-care products (Rocchetti et al., 2018).

In terms of numbers, the *O. ficus-indica* cultivation has developed in at least 18 countries and extends for more than 100,000 ha (naturalized plants or plants cultivated for home consumption not included) (Inglese et al., 2002). Mexico is the world’s largest producer of prickly pears, accounting for 45% of world production, followed by Italy (12.2%) and South Africa (3.7%) (Inglese et al., 2018; Reyes-Agüero et al., 2013). In Mexico, the planted area covers around 50,000–70,000 ha, and the gross production is around 300,000–500,000 tons per year. The prickly pear cactus is the sixth fruit crop of the country, and about 20,000 families make a profit from its cultivation, especially in those areas where few other crops can be produced (Timpanaro & Foti, 2014). Italy is the second world producer and the principal world exporter, with 7000–8300 ha of intensive plantations producing about 78,000–87,000 tons of fruits per year (Timpanaro & Foti, 2014). Other producing countries are South Africa (1500 ha, 15,000 tons per year), Argentina (800 ha, 7500 tons per year), Chile (934 ha), Peru (5000 tons per year), and USA (120 ha) (Inglese et al., 2018; Reyes-Agüero et al., 2013). In Mexico, the average fruit production is valued at around 1280 euros per ha, whereas in Italy, it is priced at approximately 1658.88 euros per ha (Basile et al., 2000; Timpanaro & Foti, 2014; Losada et al., 2017). The difference of prices depends mainly on the fruit availability (i.e., out-of-season crop) and quality, geographical origin, and not least method of cultivation, and offers a significant potential of food fraud, especially in the market scenario globalization. For example, prickly pears from Latin America may be labeled as the best quality Italian fruits and generate a greater revenue at the expense of the final consumer. As a result, the scientific community has proposed different traceability systems overtime to counteract mislabeling incidents and similar falsifications and protect the safety and quality of the commercial *O. ficus-indica*, focusing particularly on its fruits and derived products as well.

## 2.2 Chemical Characterization of *O. ficus-indica*: Towards the Geographical and Botanical Traceability

In the last decade, several scientific efforts have been devoted to the characterization of *O. ficus-indica* fruits' nutritional profile and derived products coming from different regions or belonging to diverse cultivars by exploiting a variety of analytical tools and, in some cases, univariate or bivariate statistics. Even if these works could not be properly defined as “traceability studies”, as they did not contemplate the use of multivariate statistics and did not aim to authenticate the products under investigation, they still represent a valid scientific milestone in the process of building up traceability models for the prickly pear cactus.

De Wit et al. (2010) studied the effect of variety and location on *O. ficus-indica* fruits' quality. To this purpose, three South African sites were considered, namely Free State, East Cape, and Western Caper, and 12 edible varieties common to all three sites, namely Algerian (dark pink), Gymno Carpo (orange), Meyers (dark pink), Morado (white), Nudosa (red/orange), Robusta × Castillo (orange), Roedtan (orange), Skinners Court (white/green), Tormentosa (orange), Turpin (orange), Van As (white) and Zastron (white), were investigated. Parameters, such as fruit mass, pulp percentage and peel percentage, total soluble solids, pH, ascorbic acid, titratable acidity (% citric acid), sucrose, fructose, and glucose, were determined. A basic statistical approach built on an analysis of variance (ANOVA) of each location, and a combined ANOVA of the pooled data of all the parameters from the different locations, was carried out. Obtained data pointed out that the differences of most parameters investigated at the three localities were highly significant. Highly significant differences for the measured characteristics were observed, not only for the variable “provenance”, but also for the variables “genotype” and “interaction between locality and genotype”. Hence, it was demonstrated that the *O. ficus-indica* fruits could be statistically differentiated in terms of genotype and provenance independence of several chemical and compositional traits (de Wit et al., 2010).

In 2011, Matthäus and Özcan determined the fatty acid composition and tocopherols content of prickly pear seed oils from 25 production areas of Turkey by exploiting gas chromatography coupled to a flame ionization detector (GC-FID) and high-performance liquid chromatography (HPLC) coupled to a fluorescence spectrophotometer. Concerning the fatty acid composition, major fatty acids were palmitic acid, comprised between 10.6% (location: Mut) and 12.8% (location: Kepez), oleic acid, varying between 13.0% (location: Hatay) and 23.5% (location: Kepez), and linoleic acid contents ranging from 49.3% (location: Kepez) to 62.1% (location: Hatay). Concerning tocols, only the  $\gamma$ -tocopherol was determined in the different samples, and its content changed considerably among the different areas (3.9–50 mg/100 g). Although the statistical analysis was not carried out, the authors pointed out that the nutritional profile of the prickly pear seed oil was affected by the place of origin when considering certain fatty acids and  $\gamma$ -tocopherol, as these phytochemicals may be notoriously influenced by extrinsic and intrinsic factors,

such as climatic conditions, soil type, genetic variety and cultivation method (Kritiotti et al., 2018; Mikrou et al., 2020).

In 2013, Dehbi and colleagues investigated the total phenol contents and betalains, such as betacyanins and betaxanthins, of juices from prickly pears fruits, belonging to nine Moroccan cultivars. Despite the lack of statistical analysis, these components were affected by the plant's cultivar. Indeed, the total phenolic contents varied from 354.3 µg gallic acid equivalents (GAE)/g of juice (Ait Baamran cultivar) to 643.6 µg GAE/g (Khouribga cultivar). Wide differences in terms of betaxanthins were also observed: the cultivars Alkalaa and Ait Baamrane contained the highest amounts (42.8 mg/L and 51.3 mg/L), whereas Doukkala cv. and Red Khouribga cv. were characterized by the lowest contents (18.2 mg/L and 15.8 mg/L).

In 2014, Abdel-Hameed and coworkers investigated different compositional aspects of *O. ficus-indica* fruits (juices) and peels belonging to the red and yellow cultivars and originating in the Taif governorate (Saudi Arabia). Considering the fruit juices, the estimation of total phenolics and flavonoids was performed by spectrophotometry, single polyphenols and sugars were elucidated by HPLC coupled respectively with an ultraviolet detector, and refractive index detector, minerals such as Na and K were determined by flame emission spectrophotometer (FES), whereas trace elements, such as Fe and Cu, by atomic absorption spectrometry (AAS). The cultivars characterized by red and yellow pulps differed for total polyphenol (1065 and 667 mg GAE/100 mL juice) and total flavonoid (159 and 80 mg rutin equivalents/100 mL juice) contents. A remarkable variability was pointed out also for polyphenols, such as gallic acid (452 and 748 µg/100 mL), catechin (305 and 491 µg/100 mL), rutin (2234 and 516 µg/100 mL), and kaempferol 3-O-β-D-glucoside (152 and 481 µg/100 mL). On the other hand, similar values in both red and yellow cultivars were observed for sugars, such as fructose and glucose (<3.5 g/100 mL), and investigated minerals (Na: 5.04 and 4.25 mg/100 mL; K: 20.8 and 22.2 mg/100 mL; Cu: 227 and 204 µg/100 mL; Fe: 226 and 205 µg/100 mL). Overall, although a simple descriptive statistic was performed, this study highlighted that certain phytochemicals (i.e., polyphenols) were able more than others (i.e., sugars and inorganic elements) to differentiate these prickly pear cultivars.

Khatabi et al. (2016) dealt with juices obtained from two Moroccan cultivars of prickly pears (i.e., the Moussa yellow variety and the El Akri red crimson variety). In terms of indicaxanthin and betaxanthin, the levels of betalains were determined spectrophotometrically, compared with data on pigments of prickly pears from Italy and Spain dating back to 2000–2003. In the red cultivar, betalains varied depending on the geographical provenance. Indicaxanthin ranged from 51.2 mg/kg of juice (Italy) to 190 mg/kg (Spain), whereas betaxanthin, from 36.1 mg/kg (Italy) to 300 mg/kg (Spain). Conversely, in the yellow cultivar, only the betaxanthin content was considerably different among prickly pears of different origins, as it varied between 37.8 mg/kg (Morocco) and 250 mg/kg (Spain). Even without a proper statistical analysis, the authors highlighted that the differences in betalain levels among regions were probably due to variability in the cactus ecotype, physiology, and growth conditions (Khatabi et al., 2016).

In another study, Bouzoubaâ et al. (2016) spectrophotometrically investigated the total phenolics, flavonoids and betalains, betaxanthins, and betacyanins the fruit pulp of two cultivars named Achefri and Amouslem, coming from the Moroccan regions of Tiznit and Ait Baha. Overall, these analytes varied more independence of the region of origin than cultivar. For example, total betalains were equal to 28 $\mu$ g indicaxanthin equivalents (INDE)/g and 35.3 $\mu$ g INDE/g respectively in the fruits of Achefri and Amouslem cultivars from Tiznit; while they amounted to 84.2 $\mu$ g INDE/g and 87.7 $\mu$ g INDE/g, respectively in Achefri and Amouslem cultivars from Ait Baha. Hence, it could be argued that these analytes may be more effective in differentiating prickly pears with different origins than cultivars.

Finally, a recent work by Belviranlı et al. (2019) dealt with prickly pears (dried pulp and seeds) from five different Turkish locations (Adana, Alanya, Anamur, Fethiye, and İskenderun) and investigated the effect of geographical origin on a variety of chemical variables. For the pulp, total phenolics were spectrophotometrically analyzed,  $\beta$ -carotene, ascorbic acid, and single polyphenols were determined by HPLC coupled to a photodiode array (PDA) detector, while minerals were screened by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The oil obtained from seeds was instead elucidated for its fatty acid composition by GC-FID. All the data, expressed on a dried weight basis, were statistically elaborated using one-way ANOVA followed by the post-hoc Duncan multiple comparison test.

Considering the fruit, the variables most affected by the geographical origin of *O. ficus-indica* were the total phenolic content (range: 490.7–932.8 mg GAE/100 g,  $p < 0.05$ ),  $\beta$ -carotene (40.9–130 $\mu$ g/kg,  $p < 0.05$ ), and ascorbic acid (124–240 mg/kg,  $p < 0.05$ ). For inorganic elements, P (174–403 mg/kg,  $p < 0.05$ ), K (1908–3981 mg/kg,  $p < 0.05$ ), Ca (228–1224 mg/kg,  $p < 0.05$ ), Fe (22.6–30.4,  $p < 0.05$ ), were significantly different among the samples of different provenance. Single polyphenols, such as gallic acid (0.86–166 mg/kg,  $p < 0.05$ ), quercetin (2.26–7.88 mg/kg,  $p < 0.05$ ) and isorhamnetin (1.31–7.23 mg/kg,  $p < 0.05$ ) widely varied depending on the origin of fruits. The differences in total and single phenolics,  $\beta$ -carotene, and ascorbic acid were probably related to the different geopedoclimatic contexts of the production areas (de Wit et al., 2010; Belviranlı et al., 2019). Concerning the seed oil, oleic acid (13.6–15.4%,  $p < 0.05$ ) and linoleic acid (60.9–63.3%,  $p < 0.05$ ) stood out in the fatty acid composition. Similar to what was reported by Matthäus and Özcan (2011), the differences in some fatty acid levels among investigated oil samples may be attributed to different environmental conditions, climates, and cultivation methods characterizing the origin sites.

Overall, the studies reviewed so far emphasize the concept that the analytical and when present- statistical evaluation of certain chemicals naturally present in prickly pears has been beneficial for differentiating *O. ficus-indica* cultivar or geographical provenance. Phytochemicals, such as polyphenols, betalains, minerals, and fatty acids, may be defined as “traceability markers”, i.e., substances that take part in the plant product’s composition and are naturally characterized by discriminating power (Montealegre et al., 2010). Every work dealing with the geographical or botanical traceability of plant products, including the ones deriving from

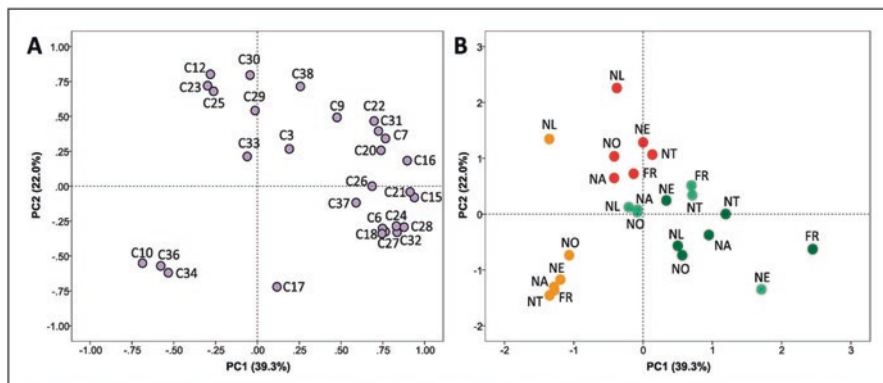
*O. ficus-indica*, contemplates selecting proper traceability markers and, thus, the analytical technique reliably characterizes them. This being established, it is imperative moving from univariate or bivariate techniques to the multivariate approach, as it is intended to treat datasets containing any number of variates and offer a much better outlook of sample discrimination concerning standard tools.

### 2.3 Geographical and Botanical Traceability of *O. ficus-indica*

The first study contemplating the use of advanced analytical techniques in combination with chemometrics is relatively recent and dealt with the elucidation of phytochemicals, such as polyphenols and betalains, in four botanical parts (young and adult cladodes, fruit pulp, and skin) of six Spanish cultivars of *O. ficus-indica* by exploiting ultra-high-performance liquid chromatography coupled to linear ion trap mass spectrometry (UHPLC-MS<sup>n</sup>) (Mena et al., 2018). In this work, the statistical analysis consisted of (1) one-way ANOVA followed by a post-hoc Tukey's honestly significant difference (HSD) test, for comparing the phytochemical composition of each botanical part among cultivars, (2) Bonferroni post-hoc test, for assessing the investigated effects (botanical part, cultivar, and the interaction of botanical part×cultivar) on the phytochemical composition, and (3) PCA, for exploring differences, as well as similarities, among the different cultivars and prickly pear parts independence of the phytochemical profile. The one-way ANOVA combined with Tukey's HSD test demonstrated that, in every aerial part, polyphenols were the phytochemicals with the greatest variability among cultivars. The Bonferroni test highlighted statistically significant effects ( $p < 0.001$ ) of variables such as aerial part, cultivar, and interaction aerial part × cultivar on the content of polyphenols (Mena et al., 2018).

Results of the PCA analysis are reported in Fig. 21.1. Two principal components (PCs) explained 61.3% of the total sample variability, being PC1 and PC2 axes representative respectively of 39.3% and 22% of the total variance. In the loading plot (Fig. 21.1a), polyphenols, such as isorhamnetin derivatives, quercetin derivatives, kaempferol derivatives, and a ferulic acid derivative, showed the highest differentiating power, as they were positively correlated to the PC1 axis. On the other hand, the score plot revealed that *O. ficus-indica* samples clustered according to the investigated botanical part rather than cultivar (Fig. 21.1b) (Mena et al., 2018). Hence, it could be concluded that the selected compositional markers were not suitable for tracing -on an exploratory basis- the botanical origin of the Spanish opuntias. Instead, they could differentiate the different botanical parts of the prickly pear cactus, probably due to the selective and peculiar synthesis of phenolics occurring in every plant part (Mena et al., 2018).

Karabagias et al. (2019b) focused on several prickly pear juices obtained from a wild cultivar of *O. ficus-indica* grown in three Greek regions (i.e., East Messinia, West Messinia, and Lakonia). These juices were elucidated for inorganic elements by inductively coupled plasma optical emission spectrometry (ICP-OES), and



Retrieved from Food Research International, vol. 108, Mena, P., Tassotti, M., Andreu, L., Nuncio-Jáuregui, N., Legua, P., Del Rio, D., & Hernández, F. "Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSn metabolomics profiles and their chemometric analysis", pp.301-308, Copyright (2018) with permission from Elsevier.

**Fig. 21.1** PCA analysis conducted on different aerial parts of *O. ficus-indica* belonging to six Spanish cultivars. (a) loading plot indicating the distribution of analyzed phytochemicals along the PC1 and PC2 axes (C# indicates the compound number referred by Mena et al., 2018). (b) score plot showing the natural grouping of samples in the component space. Dark green circles represent the old cladodes, light green ones the young cladodes, red the fruit skins, and orange the fruit pulps. "NA", "NE", "NO", "NT", "FR" and "NL" are the abbreviations of the six Spanish cultivars investigated. PC stands for the principal component. Retrieved from Food Research International, vol. 108, Mena, P., Tassotti, M., Andreu, L., Nuncio-Jáuregui, N., Legua, P., Del Rio, D., & Hernández, F. "Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSn metabolomics profiles and their chemometric analysis", pp.301–308, Copyright (2018) with permission from Elsevier

aromas by headspace solid-phase microextraction (HS-SPME) coupled to GC-MS (HS-SPME/GC-MS). For the statistical analysis, the comparison of minerals or volatiles in juices from different locations was performed by the multivariate analysis of variance (MANOVA). Quality criteria, such as the Pillai's trace and Wilks'  $\lambda$  indices, were computed to evaluate the potential of such variables of discriminating samples' geographical origin. For the Pillai's trace test, the index ranges from 0 to 1, where a value close to 1 means that the variable is contributing more to the statistical model. For the Wilks'  $\lambda$  test, the index can vary from 0 to 1 too. However, in this case, the closer to zero the value is, the more the variable contributes to the model. Finally, a LDA was applied to those variables (minerals and volatiles) selected by MANOVA to discriminate the prickly pear juices according to the geographical origin.

The Pillai's trace and Wilks'  $\lambda$  values resulting from the MANOVA on inorganic elements (respectively, 1.694 and 0.022, with a statistical significance at  $p < 0.001$ ) and volatiles (respectively, 1.938 and 0.000, with a statistical significance at  $p < 0.001$ ) demonstrated that both variables significantly varied independence of the production area of the fruit. Additionally, 7 of the 16 inorganic elements detected by ICP-OES (i.e., Na, Mg, K, Ca, P, Zn, and Ni) and 21 of the 25 volatile compounds revealed by GC-MS resulted statistically significant ( $p < 0.05$ ) for the geographical

discrimination of prickly pear juices, and, thus, were selected for respective LDA analyses (Karabagias et al., 2019b).

Based on selected inorganic elements and volatiles, the LDA models were trained with all the considered juice samples and subsequently validated, with the same samples, by a cross-validation procedure. Also, every model was described by two functions, whose statistical parameters are reported in Table 21.1. The model based on inorganic elements was characterized by good correlation values (function 1: 0.905, function 2: 0.807), and low Wilks'  $\lambda$  values (function 1: 0.063, function 2: 0.348), with a statistical significance at  $p < 0.001$ . On the other hand, the model based on volatiles consisted of two functions with even higher correlation values (function 1: 0.995, function 2: 0.947), and even lower Wilks'  $\lambda$  values (function 1: 0.001, function 2: 0.103), with a statistical significance at  $p < 0.001$ .

Results from the LDA analyses are illustrated in Fig. 21.2. Overall, juice samples clustered in three groups corresponding to the different origin sites, when the discrimination occurred both by inorganic elements and volatiles. However, much more defined clusters were defined in the space spanned by the F1 and F2 axes when volatiles was considered discrimination variables (Fig. 21.2b). Coherently, the rates of correct discrimination of original grouped samples and cross-validated samples were respectively 94.3% and 85.7% when considering inorganic elements, and 100% and 88.9% for aroma compounds (Karabagias et al., 2019b).

In another concomitant study, Karabagias et al. (2019a) characterized the same type of samples (i.e., prickly pears juice coming from the three Greek regions) for physicochemical parameters, such as acidity, vitamin C, pH, electrical conductivity, NaCl, total dissolved solids, specific weight, total sugar content, and colour coordinates ( $L^*$ ,  $a^*$ ,  $b^*$ ), and bio-functional properties, such as in vitro antioxidant activity and total phenolic content. The statistical approach was the same as described above. First, MANOVA was applied to the whole data set, and the variables characterized by a greater discriminating power were selected for the subsequent LDA. In this case, all the 13 investigated parameters resulted significant ( $p < 0.05$ ) for the differentiation of prickly pear juices according to geographical origin, and, thus, they were all subjected to LDA. The LDA model was built up using all available samples and cross-validated. It was described by two discriminant functions, whose statistical parameters are reported in Table 21.2.

Results from the LDA analysis are reproduced in Fig. 21.3. When considering physicochemical and bioactivity properties as discrimination variables, juice samples clustered into three groups corresponding to the different origin sites (Fig. 21.3). Additionally, such an LDA model allowed to correctly discriminate both original and cross-validated juice samples with a 95.8% and 81.3% rate, respectively.

Based on the described results, the studies conducted by Karabagias et al. (2019a, b) pointed out that specific phytochemicals (i.e., inorganic elements and volatile compounds), and peculiar physicochemical and bioactivity parameters as well, provided satisfactory discrimination of samples according to the geographical origin when subjected to LDA. Hence, these variables may be proposed as "traceability markers" of the prickly pear juice from the Peloponnese Peninsula. Nevertheless, a flaw of both works is that the LDA was not properly conducted. As already described

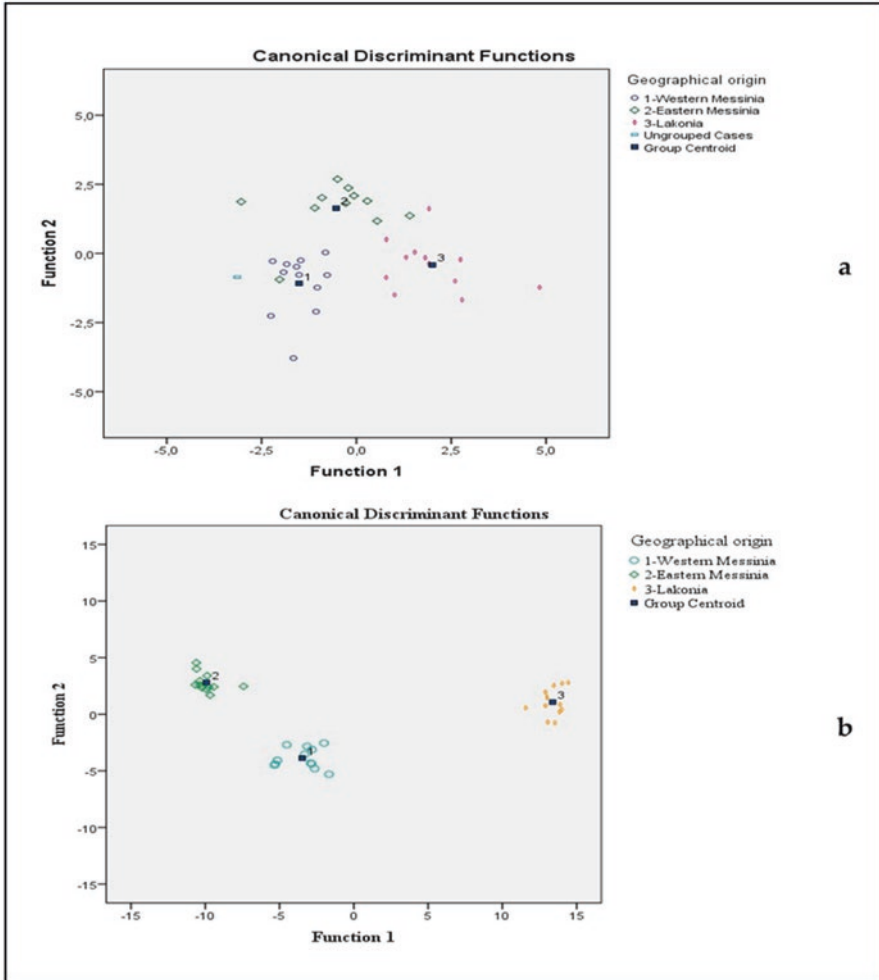


**Table 2I.1** Statistical parameters of the linear discrimination functions defining the LDA models based on inorganic elements and volatiles of the Greek prickly pear juices

LDA model	Function	Eigenvalue	Explained variance (%)	Total variance (%)	Correlation coefficient	Wilks' $\lambda$	$\chi^2$	df	<i>p</i> -value
Inorganic elements	F1	4.552	70.8	100	0.905	0.063	80.320	14	<0.001
	F2	1.874	29.2		0.807	0.348	30.611	6	<0.001
Volatiles	F1	105.215	92.3	100	0.995	0.001	159.643	42	<0.001
	F2	8.733	7.7		0.947	0.103	52.338	20	<0.001

$\chi^2$  chi-square, *df* degrees of freedom

Data retrieved from Foods, vol.8, Karabagias, V.K., Karabagias, I.K., Louppis, A., Badeka, A., Kontominas, M.G., & Papastefanou, C. "Valorization of prickly pear juice geographical origin based on mineral and volatile compound contents using LDA", pp. 123–138, Copyright (2019) by the authors. This material is reported under the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>)



Retrieved from Foods, vol.8, Karabagias, V.K., Karabagias, I.K., Louppis, A., Badeka, A., Kontominas, M.G., & Papastephanou, C. "Valorization of prickly pear juice geographical origin based on mineral and volatile compound contents using LDA", pp.123-138, Copyright (2019) by the authors. This material is reported under the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

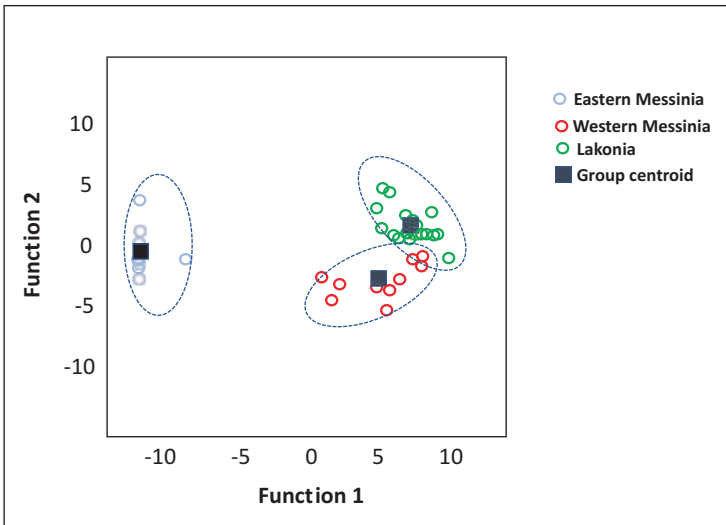
**Fig. 21.2** Scatter plots from LDA analysis illustrating (a) the discrimination of prickly pear juice according to geographical origin based on 7 minerals, and (b) the discrimination of prickly pear juice according to geographical origin based on 21 volatiles. Retrieved from Foods, vol. 8, Karabagias, V.K., Karabagias, I.K., Louppis, A., Badeka, A., Kontominas, M.G., & Papastephanou, C. "Valorization of prickly pear juice geographical origin based on mineral and volatile compound contents using LDA", pp.123–138, Copyright (2019) by the authors. This material is reported under the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>)

**Table 21.2** Statistical parameters of the linear discrimination functions define the LDA model based on the Greek prickly pear juices’ physicochemical and bio-functional properties

Function	Eigenvalue	Explained variance (%)	Total variance (%)	Correlation coefficient	Wilks’ $\lambda$	$\chi^2$	df	p-value
F1	67.384	97.5	100	0.992	0.006	202.031	24	<0.001
F2	1.603	2.5		0.785	0.384	37.784	11	<0.001

$\chi^2$  chi-square, *df* degrees of freedom

Adapted from Journal of Food Science and Technology, vol. 56, Karabagias, V. K., Karabagias, I. K., Gatzias, I., & Riganakos, K. A. “Characterization of prickly pear juice by means of shelf life, sensory notes, physicochemical parameters and bio-functional properties”, pp. 3646–3659, Copyright (2019) by Springer Nature



**Fig. 21.3** Reproduction of the scatter plot from LDA analysis showing the discrimination of prickly pear juice according to the geographical origin based on several physicochemical and bio-functional parameters. Drawn ellipses suggest the natural grouping of samples in the discriminant space. Adapted from Journal of Food Science and Technology, vol. 56, Karabagias, V.K., Karabagias, I.K., Gatzias, I., & Riganakos, K.A. “Characterization of prickly pear juice by means of shelf life, sensory notes, physicochemical parameters and bio-functional properties”, pp.3646–3659, Copyright (2019) by Springer Nature

in Paragraph 1, LDA is a classification tool relying on supervised discrimination. In other words, the LDA model is typically composed of one or more discriminant functions generated from samples of known group membership (the so-called “training set”). However, in the next step -not present in both studies- the discriminant functions shall be applied to a new sample set of unknown group membership for testing their effectiveness (the so-called “test set”). Only then the unknown group membership of the test set may be well defined, and a proper sample classification may be conducted.

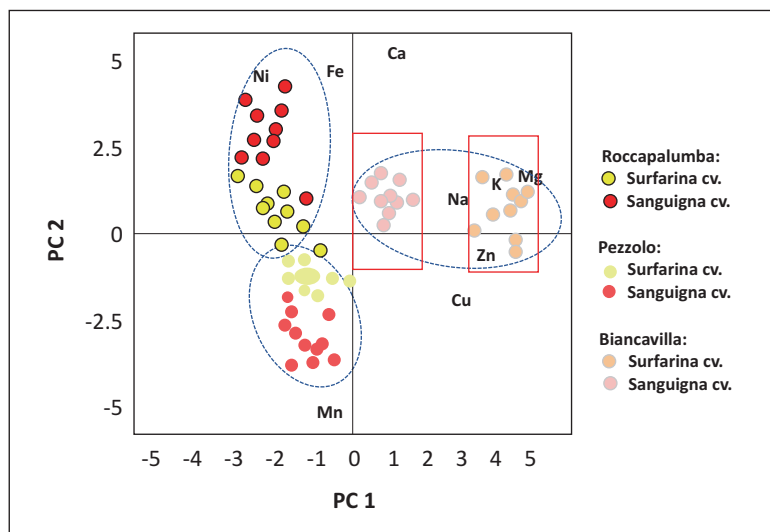
### 3 Authentication of the Sicilian PDO Prickly Pear

Accounting for nearly all the Italian production (96%), Sicily -the major southern island of Italy- has established itself as one of the major world producers and exporters of prickly pears characterized by a high-quality and an extreme delicacy (Inglese et al., 2002). A variety of Sicilian areas are intended for the cultivation of opuntias. However, the three major production sites are the southwestern foothills of the Etna volcano, Santa Margherita del Belice district (Agrigento province), and San Cono district (Catania province) (Inglese et al., 2002). Interestingly, the prickly pear produced in San Cono is protected by the PDO (protected designation of origin) logo (Ministry of Agricultural, Food and Forestry Policies, 2010); whereas the prickly pear coming from Roccapalumba, a minor production area belonging to the Palermo province, has been included in the list of the Italian traditional agri-food products (TAP) (Italian Ministerial Decree, 2000).

To guarantee the provenance and quality of those commercial fruits protected by PDO and TAP logos and, at the same time, safeguard producers and consumers, two recent studies focused on the development of traceability models for the Sicilian prickly pear (Mottese et al., 2018b; Albergamo et al., 2018).

Mottese et al. (2018b) employed ICP-MS to elucidate the element profile of Sicilian prickly pears coming from three locations, namely Roccapalumba (Palermo province), Biancavilla (Catania province), and Pezzolo (Messina province). For each location, fruits belonging to two autochthonous cultivars (Surfarina and Sanguigna) were equally considered. Then, the statistical analysis was conducted through (1) the nonparametric Kruskal-Wallis test, for evaluating the statistically significant differences among prickly pear samples of different origin, and (2) PCA analysis, for exploring differences, as well as similarities, among the samples coming from different areas, independence of their element profile.

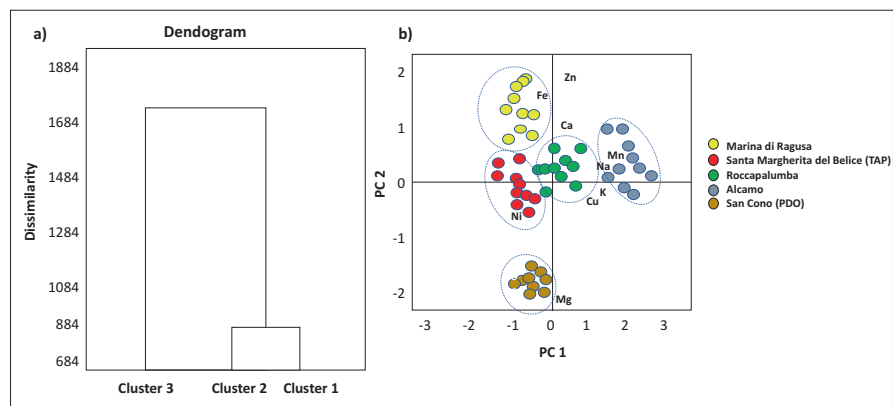
The Kruskal-Wallis test confirmed that inorganic elements significantly varied depending on the geographical origin of fruits. Considering PCA, most of the sample variability was described by the first two PCs, accounting for 72.03% of the total variance (PC1: 45.51% and PC2: 26.52% of the total variance). Results of the PCA analysis are reported in Fig. 21.4. According to the diverse sites of origin, the PCA biplot (loading + score plots) showed a natural clustering of prickly pears in the component space. The two investigated cultivars were not differentiated in fruits from Roccapalumba and Pezzolo. Nevertheless, when considering the area of Biancavilla, prickly pears from Sanguigna cv. separately grouped from the ones belonging to Surfarina cv. (Fig. 21.4). Overall, fruits from Biancavilla correlated positively with Na, K, Mg, and Zn on the PC1 axis, especially in samples belonging to Surfarina cv. Also, Mn correlated positively with fruits from Pezzolo, while elements such as Fe and Ni clustered in correspondence of prickly pears from Roccapalumba along the PC2 axis. Hence, it could be concluded that such elements served as “traceability markers” for differentiating -on an exploratory basis- the Sicilian prickly pear according to the provenance.



**Fig. 21.4** Reproduction of the PCA biplot (loading + score plots) obtained from data on inorganic elements of Sicilian prickly pears. Drawn ellipses and squares suggest the natural grouping of samples according to the production areas and the cultivar, respectively. PC, principal component. Adapted from Journal of the Science of Food and Agriculture, vol. 98, Mottese, A.F., Naccari, C., Vadalà, R., Bua, G.D., Bartolomeo, G., Rando, R., Cicero, N., & Dugo, G. (2018). “Traceability of *Opuntia ficus-indica* L. Miller by ICP-MS multi-element profile and chemometric approach”. pp. 198–204, Copyright (2017) by Society of Chemical Industry

Subsequently, Albergamo et al. (2018) investigated by ICP-MS the element profile of Sicilian prickly pears belonging to the Muscaredda cv. and coming from five geographical areas: Alcamo (Trapani province), Roccapalumba (Palermo province), Santa Margherita del Belice (Agrigento province), San Cono (Catania province) and Marina di Ragusa (Ragusa province). In this study, an approach based on unsupervised and supervised tools was performed to build up a reliable statistical model to classify the fruits according to their provenance. Specifically, a Kruskal-Wallis test followed by the post hoc Tukey’s HSD test was applied to confirm that inorganic elements of prickly pear samples varied significantly over the different Sicilian zones. Then, unsupervised HCA and PCA were employed to look for differences and similarities among samples and check for outliers.

HCA failed in the correct sample grouping, as prickly pears from the five production sites were separated only into three clusters (Fig. 21.5a). Indeed, the dendrogram reported a first and a second cluster composed respectively by samples from Alcamo and Santa Margherita del Belice, and the third cluster including fruits from other areas, namely San Cono (PDO), Roccapalumba (TAP), and Marina of Ragusa (Fig. 21.5a). The incorrect clustering probably occurred because HCA had not enough power to differentiate fruits characterized by similar contents of trace metals, such as Ni, Fe, Cu, Zn (Albergamo et al., 2018).



**Fig. 21.5** (a) Reproduction of the HCA dendrogram performed with elemental fingerprints of prickly pears from different Sicilian production areas and illustrating samples' grouping in three final clusters, (b) Reproduction of the PCA biplot (loading + score plots) obtained from data on inorganic elements of Sicilian prickly pears. Drawn ellipses suggest the natural grouping of samples according to the production areas. PC, principal component. Adapted from Journal of Food Science, vol.83, Albergamo, A., Mottese, A. F., Bua, G. D., Caridi, F., Sabatino, G., Barrega, L., Costa, R., & Dugo, G. "Discrimination of the Sicilian prickly pear (*Opuntia ficus-indica* L., cv. Muscaredda) according to the provenance by testing unsupervised and supervised chemometrics", pp. 2933–2942. Copyright (2018) by Institute of Food Technologists®

PCA resulted more suitable than HCA for a starting exploration of the data set and provided insights on the natural grouping of investigated samples. Similar to what was reported by Mottese et al. (2018b), most of the sample variability independence of the element fingerprint was described by the first two PCs, namely PC1 and PC2, accounting respectively for 42.38% and 25.06% of the total variance. Except for prickly pears from San Cono (PDO) and Marina di Ragusa differentiating along the PC2 axis, most samples plotted on PC1 (Fig. 21.5b).

Fruits from Alcamo clustered between the first and fourth quadrant, marked by the highest levels of Cu, Na, Mn, and K, and the lowest Ni content. Conversely, samples from Santa Maria del Belice grouped between the second and third quadrant and were characterized by a positive correlation with Ni and the lowest Cu and K contents. PDO prickly pears collected in San Cono (third quadrant) were marked by the highest Mg concentration and the lowest values of Fe and Zn. On the other hand, according to the biplot, prickly pears produced in Marina di Ragusa (second quadrant) were distinguished by a strong positive correlation with Fe (Albergamo et al., 2018).

A stepwise canonical discriminant analysis (CDA) and a PLS-DA were conducted to set up satisfactory classification models concerning the multivariate supervised techniques. Hence, all fruit samples were split into the training (80% of total samples) and test (20% of total samples) sets.

The stepwise CDA allowed deriving a discriminant function to maximize samples' differences among the sites of origin. Such function was characterized by a

**Table 21.3** Statistical parameters of the discrimination function define the CDA model based on the Sicilian prickly pears' element fingerprints

Function	Eigenvalue	Explained variance (%)	Total variance (%)	Correlation coefficient	Wilks' $\lambda$	$\chi^2$	df	<i>p</i> -value
F1	4995.4	100	100	0.997	$0.75 \times 10^{-10}$	38.9	24	<0.05

$\chi^2$  chi-square, *df* degrees of freedom

Adapted from Journal of Food Science, vol. 83, Albergamo, A., Mottese, A. F., Bua, G. D., Caridi, F., Sabatino, G., Barrega, L., Costa, R., & Dugo, G. "Discrimination of the Sicilian prickly pear (*Opuntia ficus-indica* L., cv. Muscaredda) according to the provenance by testing unsupervised and supervised chemometrics", pp. 2933–2942. Copyright (2018) by Institute of Food Technologists®

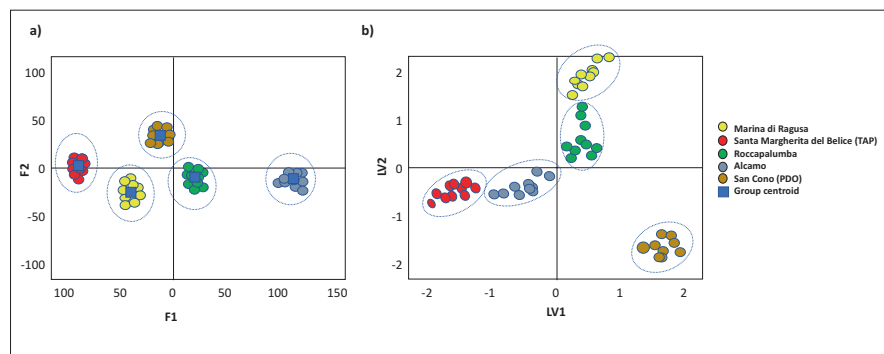
high correlation value (0.997), and a very low value of Wilks'  $\lambda$  ( $0.748 \times 10^{-10}$ ), with a statistical significance at  $p < 0.05$  (Table 21.3). Additionally, the minimum number of variables (i.e., K, Ca, and Mg) required to maximize sample classification and reduce the risk of data "over-fitting", were revealed thanks to the "stepwise" criterion (Albergamo et al., 2018).

Once defined the classification model, it was calibrated and subjected to an internal leave-one-out cross-validation using the samples' training set. Subsequently, the samples from the prediction set were classified according to the provenance using the validated CDA model. The scatter plot resulting from the CDA model's training showed that 100% of the original grouped cases were correctly classified. Sample grouping occurred mainly on the first discriminant function (F1) (90.97% of the total variance). Also, group centroids (i.e., mean discriminant scores) of each production area resulted better separated on the plane determined by F1 than F2 (Fig. 21.6a).

Considering this model, optimal classification results were obtained since not only the original grouped cases (training set) and the cross-validated samples (training set) were 100% correctly classified. Additionally, the test set's geographical provenance was correctly predicted with a rate of 100% (Albergamo et al., 2018).

The PLS-DA model was built up by deriving the optimal number of latent variables (LVs), discriminating as much as possible samples of different groups. It was then calibrated, internally validated by leave-one-out cross-validation, and checked for classification using the samples constituting the test set. The variable importance interpreted the PLS-DA model in projection (VIP) scores. Briefly, a VIP score is a measure of a variable's importance in the PLS-DA model and summarizes the contribution a variable makes to the model (Chong & Jun, 2005). VIP scores ranging from 0.8 to  $\geq 1$  usually identify the most significant classification variables. For the case study, the variables selected were K (VIP score: 0.886), Ca (VIP score: 1.098), Mg (VIP score: 1.127), and Na (VIP score: 0.884), and, among them, three were corresponding to the classification variables identified by stepwise CDA (Albergamo et al., 2018).

The PLS-DA analysis's scatter plot highlighted that 100% of the original grouped cases were correctly classified according to the geographical origin during the



**Fig. 21.6** (a) Reproduction of the stepwise CDA scatter plot illustrating prickly pear samples grouped in the discriminant space according to the geographical origin, (b) Reproduction of the PLS-DA scatter plot of prickly pear samples differentiating according to the production area. In both figures, drawn ellipses emphasize the discrimination of samples according to the geographical origin. F, discriminant function; LV, latent variable. Adapted from Journal of Food Science, vol. 83, Albergamo, A., Mottese, A. F., Bua, G. D., Caridi, F., Sabatino, G., Barrega, L., Costa, R., & Dugo, G. “Discrimination of the Sicilian prickly pear (*Opuntia ficus-indica* L., cv. Muscaredda) according to the provenance by testing unsupervised and supervised chemometrics”, pp. 2933–2942. Copyright (2018) by Institute of Food Technologists®

calibration phase (Fig. 21.6b). Most of the sample variability was described by the first four LVs, with the most negative scores related to samples from Santa Margherita del Belice and Alcamo and the most positive scores related to samples from San Cono (PDO), Marina di Ragusa and Roccapalumba (TAP) (Fig. 21.6b). High correlation coefficients confirmed the performance and the validity of the model both in the training and prediction phase (0.971–0.994), which were indicative of the fit of the model. Also, very low root mean squared errors (RMSE) in the calibration (RMSEC), in the internal cross-validation (RMSECV), and the external validation (RMSEP) provided another indication of the high performance of the model generated (Table 21.4). Similarly to the stepwise CDA, the PLS-DA model correctly classified all the prediction set samples, thus confirming a prediction ability of 100% (Albergamo et al., 2018).

It may be concluded that specific minerals, such as K, Ca, and Mg demonstrated to discriminate prickly pears independence of the production areas reliably and, thus, may be reasonably defined as traceability markers. Additionally, both stepwise CDA and PLS-DA allowed to build up models characterized by optimal classification abilities and useful for verifying the unknown provenance of *O. ficus-indica* fruits (cv. Muscaredda) within the Sicilian region.



**Table 21.4** Performance of the PLS model built using four variables (Na, Mg, K, and Ca) for the prickly pears from five different Sicilian areas

Figures of merit	Production area				
	Roccapalumba	Marina di Ragusa	Santa Margherita del Belice	Alcamo	San Cono
LVs	4	4	4	4	4
$R^2c$	0.977	0.987	0.989	0.981	0.994
$R^2p$	0.971	0.985	0.986	0.979	0.990
RMSEC	0.052	0.067	0.062	0.078	0.047
RMSECV	0.056	0.070	0.068	0.083	0.051
RMSEP	0.059	0.075	0.071	0.092	0.058

LVs latent variables,  $R^2c$   $R$ -square in calibration,  $R^2p$   $R$ -square in prediction,  $RMSEC$  root mean squared error in calibration,  $RMSECV$  root mean squared error in cross validation,  $RMSEP$  root mean squared error in prediction

Adapted from Journal of Food Science, vol. 83, Albergamo, A., Mottese, A. F., Bua, G. D., Caridi, F., Sabatino, G., Barrega, L., Costa, R., & Dugo, G. "Discrimination of the Sicilian prickly pear (*Opuntia ficus-indica* L., cv. Muscaredda) according to the provenance by testing unsupervised and supervised chemometrics", pp. 2933–2942. Copyright (2018) by Institute of Food Technologists®

## 4 Conclusion and Future Perspectives

In this chapter, the advances performed in the employment of analytical and statistical tools for food authentication and the effective use of traceability models for defining the geographical and botanical origin of fruits from the species *O. ficus-indica* were described. In certain cases, prickly pears are recognized as higher quality because they derive from a well-defined geographical area or belong to a peculiar cultivar. Accordingly, they may be sold at higher prices, be legally protected by quality logos (e.g., PDO), and, thus, add value to the relative food supply chain. In this context, the development of traceability models based on the employment of advanced analytical methods and chemometric tools for the authentication of the geographical or botanical origins of prickly pears and derived food products is a more than ever actual issue and important challenge.

As reviewed, the methods allowing the analysis and verification of prickly pears' microenvironment are very promising, but the scientific community shall further study them. A serious problem may consist of constructing comparative databases containing the chemical signatures of authentic *O. ficus-indica* from different geographical locations or belonging to a specific cultivar, which could be subsequently incorporated into traceability systems. Additionally, there is the necessity to develop in the next future reliable traceability models based on nutrients not yet explored, such as betalains (for fruits), lipids and tocopherols (for seed oil), or even pollutants carried by the environment, such as pesticides and heavy metals.

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# Chapter 22

## Antidiabetic Activity of *Opuntia* spp.



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**Abstract** Diabetes is a chronic disease associated with significant morbidity and mortality. Recently, the search for appropriate hypoglycemic agents has been focused on traditional medicine plants, partly because natural products may be better treatments than currently used drugs. Many plants were reported to be useful for the treatment of diabetes. The pharmacological agents currently used to treat type 2 diabetes produce serious side effects and fail to alter the course of diabetic complications and are not safe for use during pregnancy. The prickly pear (*Opuntia ficus-indica*) is a member of the Cactaceae family and is widely distributed in Mexico,

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Latin America, South Africa, and Mediterranean area. It has been used in traditional folk medicine because of its role in treating several diseases and conditions. The shrub (*Opuntia ficus indica* L.) has an antihyperglycemic effect. From a nutritional point of view, the plant plays a double role, medicinal and food, within the framework of the prevention of diabetes in the poor and underdeveloped countries. Its chronic prescription for people with diabetes could prevent long-term complications of this disease. The advantage of natural preparation is based not only on the ability to control hyperglycemia, but also its chronic use without undesirable effects. Recently the researchers are interested more and more in the therapeutic effects of prickly pear. Analysis of prickly pear seeds' showed a significant amount of polysaccharides, cellulose, and hemicelluloses. The structure of glucuronoxylans identified in prickly pear seeds oil has exhibited antidiabetic effects. The purpose of this chapter is to evaluate the effects of *Opuntia* spp. as a natural hypoglycemic agent for the treatment of diabetes.

**Keywords** Type 2 diabetes · Prickly pear · Cactus · Cladode · Antidiabetic · Hypoglycemic

## Abbreviations

$\gamma$ -GT	Gamma-glutamyl transferase
ALP	Alkaline phosphatase
CPSO	Cactus pear seed oil
DM	Diabetes mellitus
LDH	Lactate dehydrogenase
NPDP	Nopal dry power
NPWE	Nopal water extract
ODP	<i>O. dillenii</i> polysaccharide
OFP	Post-flowering stage
POMC	<i>Opuntia monacantha</i>

## 1 *Opuntia* spp. in the Treatment of Type 2 Diabetes Mellitus

Diabetes mellitus (DM) is a chronic disease associated with significant morbidity and mortality). Type 2 diabetes contributes to the complications of other health problems, including heart disease and stroke. Synthetic hypoglycemic agents currently used to treat type 2 diabetes produce serious side effects and fail to alter diabetic complications significantly. In contrast, bioactive compounds derived from natural resources are frequently considered safe and cost-effective (Padmanabha et al., 2011).

Currently, available diabetes therapies include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides,  $\alpha$ -glucosidase inhibitors, and glinides, which are used as monotherapy in combination to achieve better glycemic regulation (Gy et al., 2005). Many of these oral antidiabetic agents have several adverse effects (Zhang and Moller, 2000). Thus, the management of diabetes without any side effects is still a challenge. The therapies of traditional medicine include medicamentous therapies, which imply the use of drugs containing plants. This last is very widespread and revet of medical importance and economic increase. In this context, more than 1200 plants were indexed to be in experiments used in the treatment of diabetes (Mangambu et al., 2014).

*Opuntia* spp. was reported to decrease hyperglycemia, mainly in the latter part of the twentieth century (Lozoya, 1999). It has been used in traditional folk medicine to treat several diseases with hypoglycemic effects (Liu et al., 2010). The flowers of *Opuntia* have mainly been employed as folk remedies for various medical purposes, including the treatment of diabetes (Ammar et al., 2015). *in vivo* studies demonstrated that OFP extract has a significant antidiabetic effect. The OFP extract also improved lipid profile, body weight, decreasing oxidative stress and hepatic function in diabetic conditions. Therefore, it might help prevent diabetic-associated complications.

## 2 Antidiabetic Effects of *Opuntia* spp. Demonstrated Via Animal Studies

Chahdoura et al. (2017) have assessed the antidiabetic potential of aqueous flower extract of *O. microdasys* in the fructose and alloxan-induced diabetes rat model. Oral administration of the extract at the dose of 200 mg/kg for 28 days could lower the levels of glucose, while hepatic marker enzymes and products, including alkaline phosphatase (ALP), lactate dehydrogenase (LDH),  $\gamma$ -glutamyltransferase ( $\gamma$ -GT), and total bilirubin were increased.

### 2.1 Antidiabetic Activity of *Opuntia monacantha*

Researchers have investigated the effects polysaccharides extracted from cladodes of *Opuntia monacantha* (POMC) on carbohydrate metabolism in streptozotocin-diabetic rats. They compare its action with dimethyl biguanide by determining blood glucose. Polysaccharides extracted from cladodes have shown beneficial effects on the improvement of lipid and blood sugar levels. Daily treatment at 100–300 mg/kg POMC for 4 weeks brought a significant decrease in blood glucose levels of diabetic rats. The level of insulin in diabetic rats was not significantly affected by the treatment of POMC and dimethyl biguanide. It was concluded that



polysaccharides reduce intestinal glucose uptake, enhance insulin sensitivity by increased uptake and peripheral use of glucose, and decreased hepatic glucose production by inhibiting gluconeogenesis and glycogenolysis (Andrade-Cetto & Wiedenfeld, 2011).

## 2.2 Antidiabetic Activity of *Opuntia fuliginosa*

Trejo-Gonzalez et al. (1996) evaluated the hypoglycemic activity of a purified extract of *Opuntia fuliginosa* cladodes in Streptozocin-diabetic rats. The results showed that glycemia and glycosylated hemoglobin were reduced in the group of combined treatment with insulin and *Opuntia fuliginosa* extract. When insulin was removed from the combined treatment, the single prickly extract maintained euglycemia in diabetic rats. The glucose response to glucose administered also showed that rats receiving the combination therapy of insulin and *Opuntia* cladodes extract for seven weeks, followed by the extract alone, rapidly adjusted blood glucose levels. The same study reports that in humans, consumption of cladode extracts resulted in weight loss. Diabetic rats receiving *Opuntia fuliginosa* extract preserved a steady body weight, while weight loss was observed in untreated diabetic rats. The hypothesis proposed by Frati-Munari et al. (1983) was that cladode extract enhances glucose use at the cellular level.

## 3 *Opuntia stricta* Cladode Extract Reduces Blood Glucose

*Opuntia stricta* (commonly called prickly pear cactus) is a natural plant that grows in some parts of Zambia. Its fruits and cladodes are commonly consumed for nutritional and medicinal purposes, including glycaemic control among some patients with diabetes mellitus. An aqueous extract of *Opuntia stricta* cladodes produced a significant reduction in blood glucose levels in alloxan-induced diabetic mice. Over a 10-day observation period, blood glucose levels in the diabetic mice treated with *Opuntia stricta* cladode extract tended to decline toward normal levels. The extract demonstrated a significant reduction in blood glucose levels, thereby controlling the DM state. This may suggest *Opuntia stricta* cladodes' potential to be studied further for possible use in DM management.

## 4 Antidiabetic Effect of *Opuntia dillenii* Polysaccharides

Zhao et al. (2011) determined the most effective hypoglycemic component of polysaccharides from *Opuntia dillenii* haw and preliminary screened the antidiabetic effects of *O. dillenii* polysaccharide (ODP)-Ia in mice with streptozotocin-induced

DM. Different kinds of ODPs - ODP-Ia, ODP-Ib, and ODP-II' were isolated using an ultrasonic extraction method and diethylaminoethyl. In another study, Trejo-Gonzalez et al. (1996) assessed the blood glucose-reducing activity of extract from prickly pear cactus (*Opuntia spp.*) in streptozotocin-induced diabetic mice. Although the mechanism of action was unknown, they reported that the major substance that reduces blood glucose was presumed to be the dietary fiber in *Opuntia* extract.

Hwang et al. (2017) classified dietary fiber into water-soluble dietary fiber and non-water-soluble dietary fiber. Water-soluble dietary fiber comprises mucus, gum, pectin, and hemicelluloses, while non-water-soluble dietary fiber composed of cellulose, lignin, and a large hemicellulose fraction. The gel formed by water-soluble dietary fiber is known to prolong the passage of food through the intestine. In their investigation of antidiabetic effect of fresh Nopal (*Opuntia ficus-indica*) in low-dose streptozotocin-induced diabetic rats fed on a high-fat diet revealed that both Nopal water extract (NPWE) and Nopal dry power (NPDP) had inhibitory activity on  $\alpha$ -glucosidase enzyme and prevented the radical increase of blood glucose levels as decomposition into monosaccharide by  $\alpha$ -glucosidase in the small intestine. Their study also verified the function of *Opuntia* aqueous extract as a hypoglycemic agent by confirming its inhibitory activity on  $\alpha$ -glucosidase enzyme *in vitro*. The foregoing evidence seems to agree with earlier evidence (Ou et al., 2001) that postulated three pathways by which bioactive constituents of *Opuntia* spp. produce antidiabetic effects. The first pathway by increasing the small intestinal content's viscosity and retarding the diffusion of glucose. Secondly, by adsorbing glucose and preventing its diffusion, and finally by inhibiting the activity of  $\alpha$ -glucosidase and postponing the release of glucose from starch. Collaborative scientific evidence is required to support *Opuntia* plant extracts' complementary use in reducing dosage requirements for conventional antidiabetic drugs such as sulphonylureas, biguanides, and insulin *via* synergistic effect.

For instance, Trejo-Gonzalez et al. (1996) found that the combination of insulin and a purified extract of *Opuntia fuliginosa* Griffiths (1 mg/kg oral dose) reduced blood glucose and glycated hemoglobin levels to normal in rats compared to high quantities of parenteral insulin required for an equivalent hypoglycaemic effect.

## 5 Antidiabetic Properties of Cactus Pear Seed Oil

Cactus pear seed oil (CPSO) is an organic extract that contains fatty acids, represented by linoleic acid, a major polyunsaturated fatty acid, oleic acid, the dominant monounsaturated fatty acid, and palmitic acid, the major saturated fatty acid (Ennouri et al., 2005; Ramadan & Morsel, 2003; Sawaya & Khan, 1982). Seed oil from this plant reduced plasma glucose levels, improved the hepatic and plasma lipid profile, and reduced muscular glycogen in healthy rats treated subchronically (Ennouri et al., 2007).

Berraaouan et al. (2014) investigated the hypoglycemic and antihyperglycemic effects of CPSO, and its possible mechanism of action. This effect should prevent

the complications of glucotoxicity and is due, in part, to inhibition of intestinal D-glucose absorption. The study shows that oral administration of CPSO prevented the diabetogenic effect of Allx. This is possibly due to the presence of antioxidant compounds, which act by inhibiting Allx-induced free radicals production and more probably by quenching them if they are produced.

A study demonstrated that the Da-tocopherol-enriched diet prevents Allx-induced diabetes in mice (Kamimura et al., 2013). However, g-tocopherol is a major vitamin E in CPSO. It has been reported that g-tocopherol has antioxidant and anti-inflammatory activities (Wagner et al., 2004). These findings were supported by Tomasch et al. which demonstrated that g-tocopherol rich oil improves plasmatic antioxidant capacity and decreases low-density lipoproteins in healthy male volunteers (Tomasch et al., 2001).

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# Chapter 23

## Anticancer Activity of *Opuntia* spp.



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**Abstract** According to the World Health Organization (WHO), cancer was ranked as the second leading cause of death. Research on natural products has recently received a lot of attention from health professionals for improving overall well-being and the prevention of diseases, including cancer. Many herbs have been screened for anticancer activity *in vitro* and *in vivo* as an alternative drug or in combination with chemotherapy. *Opuntia* spp. (prickly pear cactus) which belongs to the Cactaceae family is a xerophytic plant with 200–300 species. It is distributed worldwide and has great economic potential. *Opuntia* spp. fruits revealed to be

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promising natural sources for the production of functional products and the development of novel chemotherapeutic agents. Studies have demonstrated the cytotoxic effects of various parts of *Opuntia* (fruits), with or without peels and seeds, the cladodes or stems, and even the roots, on cancerous cell lines. The cactus pear fruit extract inhibits the proliferation of cervical, ovarian, and bladder cancer cell lines *in vitro*, and suppresses tumor growth in the nude mice ovarian cancer model *in vivo*. It was reported that the anticancer activity is mostly because of bioactive polysaccharides that induce cell apoptosis or cell cycle arrest and angiogenesis suppression with minor toxicity on normal cells. This chapter aims to study the anticancer activity of *Opuntia* spp.

**Keywords** Cactaceae · Prickly pear · Polysaccharides · Antitumor · Antiproliferative

## Abbreviations

ADP Poly (ADP) ribose polymerase  
 DNA Deoxyribonucleic acid  
 O. *Opuntia*  
 PARP Ribose polymerase  
 ROS Reactive oxygen species  
 WHO The World Health Organization

## 1 *Opuntia* spp. Derived Products as Antiproliferative Agents in Human Colon Cancer Cell Line (HT29)

Cancer is a devastating disease and treatments for recurrent and metastatic diseases remain a center of clinical attention. A potential solution to this problem lies in using plant-derived compounds or phytochemicals, which are considered pharmacologically safe and may provide a viable option for anticancer therapy. While continuing efforts have been made for discovering new molecular target-based molecules, there is an emerging interest in the chemotherapeutic application of natural substances.

*Opuntia* spp. have been characterized extensively at the biochemical level, and their biological effects, including therapeutic properties against cancer. Many studies have shown that the cactus has antiproliferative activity against a wide range of cancers (Garcia-Solis et al., 2009; Feugang et al., 2010). Kim et al. (2014) showed that extracts from *O. humifusa* cladodes induced apoptosis in MCF-7 cells and human colon SW-480 cells. The polyphenol-rich juice concentrates of *Opuntia ficus-indica* and *Opuntia robusta* inhibited cancer (human colon carcinoma HT29

cell line) cell growth and induced cell cycle arrest in different checkpoints G1, G2/M, and S, but not to Caco2 (Serra et al., 2013). Natural extracts from juice residues (peels and seeds) were reported to be more effective than juice concentrates on inducing a cell-cycle arrest in the same cells. Interestingly, this effect paralleled an increase of reactive oxygen species (ROS) in the cells, which suggests a ROS-induced cell death probably due to the extracts' pro-oxidant effects. The presence of compounds such as  $\beta$ -cyanines, flavonoids, and phenolic acids could be responsible for cell cycle arrest.

Antunes-Ricardo et al. (2014) evaluated the cytotoxic effects of *O. ficus-indica* cladode extracts of purified isorhamnetin glycosides on two models of human colon cancer cell lines (HT-29 and Caco2), representing apoptosis-resistant and apoptosis-susceptible cell lines, respectively, while normal fibroblasts (NIH 3 T3) were used as control. Authors reported that cladode extracts and isorhamnetin glycosides were more cytotoxic to HT-29 cells than to Caco2 or to control, with an effect of the glycosylation pattern.

The effect of *O. ficus-indica* fruit aqueous extract and its betalain pigment indicaxanthin on the proliferation of the human colon cancer cell line Caco2 was studied (Naselli et al., 2014). The authors revealed a dose-dependent apoptotic effect on proliferating cells, while no effect was reported on differentiated cells. In this study, indicaxanthin presented an epigenomic consequence on the tumor suppressor gene p16INK4a, through demethylation of its promoter and stimulation of its expression.

## 2 Anticancer Properties of *Opuntia humifusa* Extracts Against Human Cervical Carcinoma Cells

The uterine cervix's carcinoma is the second most common women cancer in developing countries (Alvarez-Salas et al., 2007). Current treatment, radiotherapy, and adjuvant chemotherapy have limited efficacy. A potential solution to this problem lies in using plant-derived compounds that are considered pharmacologically safe and may provide a viable option for anticancer therapy. The water extracts from *O. humifusa* have been reported to exert anticancer effects on human glioblastoma or astrocytoma, epithelial-like (U87MG) cells (Hahm et al., 2010). One strategy for cancer control is chemoprevention, which uses dietary or synthetic agents to prevent or to slow carcinogenesis. In another study, *Opuntia humifusa* extracts (Hahm et al., 2010) evaluated the inhibition of growth of U87MG human glioblastoma cells. The results showed that aqueous fractions from *O. humifusa* induce G1 arrest and non-apoptotic cell death and significant increase in ROS production in U87MG cells inhibiting U87MG human glioblastoma cell proliferation.

Cactus fruit ethyl acetate extracts containing flavonoids, *trans* taxifolin, and dihydrokaempferol also suppressed HeLa cervical carcinoma cell proliferation (at  $\geq 100\mu\text{g/mL}$  concentrations). Simultaneously, normal human BJ fibroblasts were unaffected, suggesting potential application as an intervention for human cervical

carcinoma management. The anticancer effect of *Opuntia* fruit extracts has also been reported *in vitro* using ovarian, cervical, and bladder cancer cells and *in vivo* using a nude mice ovarian cancer model.

### **2.1 Growth Inhibitory Effect of Cactus Pear Solution on Human Ovarian Cell Lines**

Results show that the cactus pear inhibited the growth of different cancer cells *in vitro* and *in vivo*. Cactus products inhibited cancer cell growth with concentrations as low as 5%. The cell cycle was also affected at this concentration with an increase in the G1 phase.

### **2.2 Extracts of *O. humifusa* Inhibit Cell Proliferation in Human Cervical Cancer Cells**

HeLa cells were treated with the extracts for 24 h at concentrations ranging from 100 to 1000  $\mu\text{g/mL}$ , and the number of viable cells was determined using the MTT-based assay. Hexane extracts of *O. humifusa* significantly suppressed cell proliferation. Additionally, treatment with the ethyl acetate extracts (100  $\mu\text{g/mL}$ ) of the fruit, stem, and root significantly decreased the proliferation of HeLa cells. The effects of *O. humifusa* extracts on cell growth were further examined using BJ cells. Treatment of the cells with the ethyl acetate extracts markedly increased the number of viable cells.

## **3 Anticancer Properties of *Opuntia ficus-indica* Induces Apoptosis in Human Chronic Myeloid Leukemia Cell Line-K562**

Betalains are water-soluble nitrogen-containing pigments that are responsible for the bright red or yellow color of fruits. One of these plants, *Opuntia ficus-indica* (L.) Mill. (cactus or prickly pear) contains betalains in the fruits (Stintzing et al., 2003). Betalains are associated with some beneficial health effects, including anticancer activity. Besides, a role for betalain pigments in the chemoprevention against lung and skin cancers has been documented (Kapadia et al., 1996). It is demonstrated that natural food colors, such as betanin, can inhibit the cell proliferation of various human tumor cells (Muntha Reddy et al., 2005). In a previous work, Sreekanth et al. (2007) reported that betanin isolated from the *Opuntia ficus-indica* fruits showed antiproliferative activity on human chronic myeloid leukemia cell line (K562)



through the intrinsic apoptotic pathway, and cell death was recorded at an inhibitory concentration (IC<sub>50</sub>) of 40 mM betanin. In particular, this compound induced cell cycle arrest in the sub G0/G1 phase and promoted apoptosis in leukemia cells.

Further studies involving scanning and transmission electron microscopy revealed apoptotic characteristics such as chromatin condensation, cell shrinkage, and membrane blebbing. Agarose electrophoresis of genomic DNA of cells treated with betanin showed fragmentation pattern typical for apoptotic cells. Flow cytometric analysis of cells treated with 40 mM betanin showed 28% of cells in the sub G0/G1 phase. Betanin treatment to the cells also induced the release of cytochrome c into the cytosol, poly (ADP) ribose polymerase (PARP) cleavage, down-regulation Bcl-2, and reduction in the membrane potentials. Confocal microscopic studies on the cells treated with betanin suggest the entry of betanin into the cells. Thus, these studies demonstrated that betanin induces apoptosis in K562 cells through the intrinsic pathway and is mediated by the release of cytochrome c from mitochondria into the cytosol and PARP cleavage. The antiproliferative effects of betanin add further value to the nutritional characteristics of *O. ficus-indica*.

#### **4 Anticancer Activity of *Opuntia* Extract on Human Breast Cancer Cell Line**

Cancer is one of the leading causes of death worldwide. In particular, breast cancer is one of the more frequent causes of premature mortality in the female population. Cancer stem cells have gained attention in the last years as responsible for tumor progression and resistance to therapy in breast cancer tumors (Ferrari et al., 2013). At the moment, chemotherapy seems to be the only possible treatment involving side effects. Therefore, great efforts are devoted to developing new strategies using therapeutic agents to improve and optimize the treatment (De la Mare et al., 2014). The result has been reported that the ethyl acetate extract of *O. humifusa* stem repressed breast cancer cell proliferation (Kim et al., 2013). *Opuntia polyacantha* alkaloid extract shows great cytotoxic activity against MCF-7 cells compared with its minor cytotoxic effect on the normal cell line, which may open an innovative study in cancer treatment as either an alternative drug or immunoadjuvant agent, especially its safety (Lubna Abdulazeem et al., 2018).

#### **5 Anticancer effect of Prickly Pear (*Opuntia ficus indica*) Juices**

It appears that *Opuntia ficus-indica* has been subject to intensive exploitation due to its great compositional diversity. Several studies have agreed that *Opuntia ficus indica* juice was rich in minerals and vitamins (MoBhammer et al., 2006; El-Gharras

et al. 2006) and may potentially be included in animal and human diets. It could be noted that the cactus fruit juice of *Opuntia ficus indica* is rich in betalains (betanin and indicaxanthin) (El-Gharras et al., 2008), and also elevated in polyphenolic flavonoids (quercetin, kaempferol, and isorhamnetin) and various carotenoids. These fruits have shown several effects, such as anticancer (Zou et al., 2005). The *in vitro* cytotoxicity was measured toward the P-815 cell line by the growth inhibition assay determined by the MTT viability assay. It was found that the juice of different cultivars exerts a dose-dependent growth inhibition against the P-815 cell line.

## 6 Anticancer Effects of Prickly Pear (*Opuntia ficus indica*) Seeds Oil

The prickly pear seed oil composition and its chemical characteristics were investigated (Salvo et al., 2002). Ramadan and Morsel (2003) compared the seed and pulp oil composition. All the authors have agreed that *Opuntia ficus-indica* seed oil is rich in polyunsaturated fatty acids (PUFA) and vitamins and may be included in animal and human diets. However, data on the nutritional value of prickly pear oil are at present unknown. This oil also had an inhibitory effect on the growth of two different types of cancer cells [Colo-205 cell line and Hepatocellular carcinoma cell line (HepG2)]. The findings of this trial highlighted the beneficial effect of *O. ficus-indica* seed oil on health.

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**Part IV**  
**Technology and Processing of *Opuntia* spp.**

# Chapter 24

## Innovative Technologies for the Identification of Chemical and Bioactive Compounds in *Opuntia* spp. Plant, Food and Waste



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**Abstract** The family *Cactaceae* (commonly known as cactus) comprises plants with a wide applications in traditional folk medicine as well as in food and pharmaceutical industries. Among the geographically spread species in Africa, America, and the Mediterranean basin, *Opuntia* spp. has received particular attention attributed to its valuable functional and therapeutic compounds. Phenolic acids, antioxidants, biopeptides, pigments, soluble fibers are among those compounds of pharmaceutical and nutritional value. This chapter aims to comprehensively review recent studies and methodologies used to identify these compounds in the various parts of the plant and the biomass wasted during cultivation and fruit processing.

**Keywords** Nutrients · Bioactive compounds · Green methodologies · Innovative technologies · Waste

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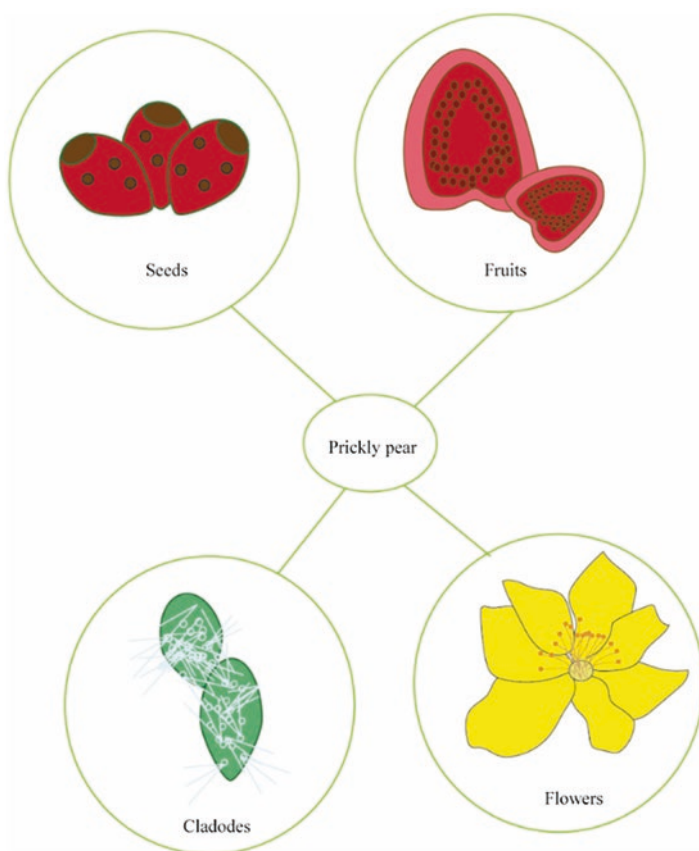
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## 1 Introduction

Various parts of *Opuntia* spp., including flowers, cladodes, seeds, and fruits (Fig. 24.1), from the family *Cactaceae*, are sources of nutrients and biologically active compounds (Ghazi et al., 2013) and have been administered as food additives for human and animal use, due to a variety of health-promoting activities (Galati et al., 2002; Barba et al., 2020).

*Opuntia* species (spp.) grow in arid and dry areas characterized by hard environmental conditions, typically in Africa, America, and in the Mediterranean (Del Socorro Santos Díaz et al., 2017). Although the differences in the bioactive components of the different species concerning their origin, several beneficial effects have been attributed to *Opuntia* spp. for treating metabolic diseases (e.g., diabetes, and obesity), cardiovascular and inflammatory conditions (Young et al., 2005).



**Fig. 24.1** Overview of prickly pear

Identifying the different bioactives and their in-depth characterization would help explain their biological and medicinal properties *in vivo*.

Numerous conventional techniques, such as lixiviation, ultrasonic-assisted extraction, supercritical fluid extraction (SFE) and microwave-assisted extraction (MAE), solid-phase processes, and high-pressure pretreatment, have been employed to extract and detect the existing bioactive compounds in *Opuntia* spp. (Aragona et al., 2018). Generally, bioactive compounds should be analyzed in an integrated and multidisciplinary approach. The joined use of innovative analytical techniques and statistical methods in food science is now of pivotal importance in driving toward the great challenge of the green transition for modeling agri-food systems.

Sampling and extraction procedures represent crucial phases in the recovery, isolation, and identification of bioactive compounds. The optimal extraction procedure should provide the maximum yield in terms of the concentration of target compounds. It is worth underline that different variables and parameters, such as pretreatment of the sample, solvent/sample ratio, type of solvent, particle sizes, time, and temperature of extraction, should be considered (Spigno et al., 2007).

Innovative methodologies have been introduced, with particular emphasis on green and sustainable techniques. Microwave-assisted extraction, supercritical fluid extraction, sub-critical fluid extraction, and ultrasound-assisted extraction are novel extraction technologies that may contribute to shorten time-processing, saving energy and solvent consumption. Ultrasound-assisted extraction (UAE) is generally a method for separating different plant-derived chemical compositions of interest exploiting solvents and acoustical energy as mechanical energy transmitted by pressure waves in a medium through the induction of vibrational molecule motion stretching and compressing alternately medium's molecular structure because of time-dependent pressure. The microwaves can heat an object's molecules assisted extraction (MAE) technique through dipole rotation and ionic conduction. The end of both methods is when the cell wall is destroyed, and the target compounds are released into the extracting solvent. A supercritical fluid (SCF) refers to a substrate that exceeds critical values of temperature and pressure ( $T_c$  and  $P_c$ , respectively), where there are no distinct liquid and gas phases. The SCF shows characteristics that are intermediate between those of a liquid and gas.

The recovery of compounds of nutritional and nutraceutical interest from food waste represents the goal of circular bioeconomy and the biorefinery concept. Hence, this chapter aims to comprehensively review the various methods employed to identify these compounds in the various parts and waste of the *Opuntia* species.

## 2 Focus on Analytical Techniques

Various new techniques to analyze the chemical compositions and identify bioactive compounds in the various parts of the plant, by-products, and waste of *Opuntia* spp. are shown in Table 24.1. A study on xoconostle fruit pulp (*Opuntia matudae Scheinvar*) found a high concentration of ascorbic acid (31.6 mg/100 g) in fresh

**Table 24.1** Different analytical approaches for the identification of bioactive compounds in *Opuntia* spp.

Part of plant	Bioactive compound	Content (mg/100 g)	Analytical approach	Reference
Fruit	Quercetin, isorhamnetin, luteolin and kaempferol	90.0, 49.4, 8.4 and 7.8 µg/g	HPLC-DAD	Fernández-López et al. (2010)
Cladodes	Piscidic acid I and isorhamnetin derivative I	967.2 and 254.4 mg/100g	HPLC with Diode Array Detector (HPLC-DAD)	Missaoui et al. (2020)
Cladodes	cyanidin, pelargonidin, petunidin, kaempferol, isorhamnetin and apigenin	1058.55, 187.97, 186.55, 241.68, 98.42 and 40.69 mg/kg of phenolic equivalents	UHPLC-ESI-QTOF-MS analysis	Rocchetti et al. (2018)
Flowers	Quinic acid, rutin (quercetin-3-O-rutinoside) and hyperoside (quercetin-3-O-galactoside) and quercetin-3-O-rhamonoside	390.27131.85 mg/100g, 390.27 mg/100g, 325.31 mg/100g and 134.12 mg/100g	LC-ESI-MS/MS	Ammar et al. (2018)

fruit pulp as well as fiber (30.1 g/100 g), phenolics (59.4 mg/mL), flavonoids (58.4 mg/mL), polyunsaturated fatty acids (80.2 g/100 g) and  $\gamma$ -tocopherol (6.43 mg of catechin equivalents (CE)/g) in the seeds (Morales et al., 2012). Surup et al. (2021) reported, for the first time, the presence of opuntisine A and B, novel cyclopeptide alkaloids, in the fruit extract of *Opuntia stricta* var. *dillenii*. In this study, the first high-performance countercurrent chromatography (HPCCC) was used to determine the plant's chemical compositions, and subsequently, off-line LC-ESI-MS/MS injections were applied to monitor the HPCCC separation. Finally, the obtained new metabolites were characterized by high-resolution mass spectrometry, derivatization experiments, and 1D/2D-NMR spectroscopy. In a study, spectrophotometrically (<sup>1</sup>H NMR analysis) detected phytochemical compositions obtained from *Opuntia littoralis* were 8-carbomethoxy-5-hydroxyl-6-methyl isoflavone in cladodes and kaempferol-3-O-rhamnoside-7-O-glucoside and luteolin in fruit juice as well as kaempferol, quercetin, gallic acid, ferulic acid, chlorogenic acid isolated from both cladode extract and fruit juice (Abd El-Moaty et al., 2020).

With respect to the characterization of the prickly pear fruit volatile fraction, one of the most innovative approaches is still that one based on enantio-differentiation and <sup>13</sup>C/<sup>12</sup>C isotope ratio analysis, performed by multidimensional gas chromatography-mass spectrometry (MDGC-MS) and on-line gas chromatography-combustion isotope ratio mass spectrometry (HRGC-C-IRMS), applied to



determine both chiral and achiral constituents (Weckerle et al., 2001). The first technique allowed to determine the enantio distribution of key constituents, such as methyl 2-methyl butanoate, methyl 3-hydroxybutanoate, 1-phenyl-ethanol, linalool,  $\gamma$ -nonalactone,  $\gamma$ -deca-lactone and  $\gamma$ -dodecalactone, which was discussed concerning data previously provided for these compounds from various plant origins. In contrast, the  $\delta^{13}\text{C}$  values measured for 1-hexanol, E-2-hexenol, E-2-nonenol, and E,Z-2,6-nonadienol were clear-cut indicators for their origin from plants with *Crassulaceae* acid metabolism, and thus could be helpful for authenticity evaluations. The same approach could help characterize the bioactive constituents of the fruit volatile fraction, such as volatile phenolic compounds, terpenes, fatty acid derivatives, and aldehydes (Zito et al., 2013).

### 3 Conclusion and Future Remark

*Opuntia* spp. is a valuable rich source of bioactive compounds with various applications in the pharmaceutical and nutraceutical industries. Because of this potential, there is a need to develop new comprehensive and innovative analytical processing approaches to promote the use of already known molecules and to discover new compounds for therapeutic and functional purposes. Nevertheless, further studies still need to reach definitive conclusions about the effective ingredients and concentrations of the well-known and newly extracted compounds of these plants. The expression and optimization of bioactive compounds of *Opuntia* spp. extracts might be improved by further understanding the metabolism of the plant and processing methods applied. Moreover, structural scrutiny of the plants' potential novel and unknown compounds requires sophisticated and advanced techniques and approaches to find possible new applications. At the same time, there is a need for further research to enhance knowledge of the safety aspects.

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# Chapter 25

## Innovation Technologies for Extracting *Opuntia* spp. Seed Oil



Maryna de Wit and Arno Hugo

**Abstract** Cactus pear seed oil is a novel and essential oil. Progressive expansion on the extraction and refining of cactus pear seed oil has been made in recent years. This was and is done to expand the market and use of cactus pear seeds in countries producing *Opuntia* spp. cactus. The seed oil is an excellent source of bio-active substances such as essential fatty acids, sterols, phenolics, tocopherols and carotenoids. It is predicted that non-traditional cold-pressed oils' use in the cosmetic and nutraceutical market will increase, and as a result, advanced extraction methods need to be explored. Conventional extraction methods generally use heat, maceration, agitation and long extraction times, e.g. solvent extraction, while microwave, supercritical fluids, e.g. CO<sub>2</sub>, and ultrasonic-assisted extraction as well as hydro-distillation are unconventional methods. These methods all exert a physical effect on the sample. This chapter will report on the effect of extraction methods on oil yield, fatty acid composition and nutraceutical properties of seed oil from various *Opuntia* spp.

**Keywords** Conventional · Chemical · Mechanical · Green · Unconventional · Vegetable oil · Pulsed-electric fields · Super-critical carbon dioxide

### Abbreviations

2-MeO	2-methyloxolane
AEE	Aqueous enzyme extraction
FA	Fatty acids
HD	Hydro-distillation

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HPAE	High-pressure assisted extraction
HPH	High-pressure homogenization
HPP	High-pressure processing
HVED	High-voltage electrical discharge
MAE	Microwave-assisted extraction
MAEE	Microwave-assisted enzymatic extraction
MAHD	Microwave-assisted hydro-distillation
PEF	Pulsed-electric fields
PEF-AE	Pulsed-electric field assisted extraction
PLE	Pressurized liquid extraction
PUFA	Polyunsaturated fatty acids
SAE	Surfactant-assisted extraction
SC-CO <sub>2</sub>	Super-critical carbon dioxide
SCFE	Supercritical fluid extraction
SD	Steam-distillation
SFE	Supercritical fluid extraction
UAE	Ultrasonic-assisted extraction
US	Ultrasound
USE	Ultrasound-assisted extraction

## 1 Introduction

Cactus pear (*Opuntia* spp.) has recently been referred to as the *Cactaceae* plant with the highest economic relevance in the world (Hernández García et al., 2020). In recent years, food researchers and the food industry have shown increasing interest in not only the *Opuntia* fruit, but also the cladodes (Barba et al., 2017). Cactus pear fruit contain phytochemicals that provide health properties and benefits, which in turn, classify these fruit and other plant parts thereof as functional food. Cactus pear fruit are mostly consumed fresh, but are also processed and preserved into products such as juices, jams, etc. (de Wit et al., 2014, 2018; du Toit et al., 2018). During processing of the fruit, large amounts of waste and by-products are produced e.g. peels and seeds. These “waste” and by-products also contain considerable amounts of functional ingredients which could generate not only value-added nutraceuticals, cosmetic and skin care products, but also an additional income by means of valorization of the waste products. According to Barba et al. (2017) the extraction of valuable compounds from the by-products is drawing a lot of attention and causes it to be on the verge of commercialization.

Cactus pears belongs to the *Opuntia* genus of which *Opuntia ficus-indica* is the most produced and promoted. Other commercially grown species include *O. stricta* and *O. dillenii* for both food and feed consumption (Barba et al., 2017). *Opuntia* spp. are mainly cultivated in arid and semi-arid regions of the world, with Mexico being the main producer, followed by Italy (Sicily) and South Africa. Other important

countries include Chilli, Argentina, Peru, Brazil, Morocco, Tunisia, Egypt, Greece, and Spain (Hernández García et al., 2020).

Cactus pear fruit consists of a thick peel (37–67% fruit mass) surrounding a juicy pulp (28–55% fruit mass) containing many seeds (2–10%). Peel and pulp colour vary from white to green, yellow, orange, red, pink and purple. These colours are ascribed to the presence of various pigments including chlorophyll, carotenoids and betalains. Carotenoids and betalains are powerful antioxidants and occur together with other biologically active nutritive and health ingredients, such as ascorbic acid, phenolics, flavonoids, amino acids including proline and taurine (Fernández-López et al., 2010; Moussa-Ayoub et al., 2011; Jiménez-Aguilar et al., 2015), minerals such as Ca, Mg, Fe, K, Na and P as well as soluble fibres (Stintzing et al., 2001). These functional ingredients are responsible for many health benefits, mainly in the ailment of metabolic syndrome.

## 2 Vegetable Oils and Its Extraction

Vegetable oils are important for food (feeding, margarine, bakery, and canned foods) and non-food (pharmaceuticals, cosmetics, detergents, and paints) applications. Vegetable oils and fats are found in plant tissue in nature, e.g. seeds, pulp, stone fruits, tubers and sprouts, i.e. oleaginous plants with oils in seeds (sunflower, soybean, and rapeseed), oleaginous fruits (olive, coconut, and palm), oleaginous tubers (peanuts) and oleaginous germ (corn) (Ionescu et al., 2013). Oils from oilseeds provide essential fatty acids, vitamins and calories, while the de-oiled cake is a valuable protein source for animals. About 2/3 of the total volume of oil products are edible oils and used in food, while the other 1/3 is non-edible oil and used in industries.

Extraction methods vary according to the plant parts' nature and oil content. The extraction methods used aim to collect the maximum of oil with minimum cost. Nowadays, worldwide the trend is to optimize these methods. Four basic methods are employed for oil extraction: chemical, supercritical fluid, steam distillation and mechanical methods.

### 2.1 Mechanical (Pressing)

Oil is separated from the oleaginous material (liquid-solid mixture) using compressive external forces achieved by machines called presses. In principle, seeds are placed between barriers whereby the volume available to seeds is reduced by pressing, causing the forcing of oil out of the seed (Nde & Foncha, 2020). Pressing could be done in hydraulic presses or in screw presses (where the pressing force is created by a helical body that rotates in a closed space), while the hydraulic oil press uses the

principle of a hydraulic ram. Hydraulic presses are nowadays replaced by continuous screw presses and continuous solvent extraction plants. To improve the oil extraction process by screw presses, variables such as applied pressure, temperature and moisture conditioning of samples, are important. To improve cooking techniques, oil recovery and oil quality, extruding-expelling technologies are adopted. This involves the coupling of a dry extruder with the continuous screw press. A new screw press design that combines pre-pressing and extruded collets formation into a single processing unit are one of the most recent developments (Ionescu et al., 2013).

## 2.2 *Chemical Extraction*

Chemical extraction methods include enzymes and solvents being used for oil extraction. During solvent extraction, a process of separation of a liquid from a liquid-solid system takes place by the use of a solvent such as light paraffinic petroleum fractions i.e. pentane, *n*-hexane, heptane and octane. Seeds are first flaked to increase the contact area of the seed with the solvent and subsequently cooked to denature cell tissues for easier penetration of the solvent into the flakes. This mixture of oil and solvent is then heated in evaporators to remove the solvent. Solvent oil extraction has certain limitations and disadvantages: solvents are harmful, flammable, initial capital and operation cost is high and energy requirement is high.

Chemical extraction using enzymes involves cooking of the seeds and soaking the cooked seeds in water. Enzymes are added to digest the solid material after which liquid-liquid centrifugation is used to separate the oil and the residual enzymes (Ionescu et al., 2013).

## 2.3 *Supercritical Fluid (SFE)*

Especially for edible oils, it is important to replace solvents with supercritical fluids (supercritical fluid extraction, SFE) in vegetable oil extraction. The solvent is a gas above its critical point. Mostly CO<sub>2</sub> is used as a supercritical fluid since it does not extract molecular oxygen and is not a toxic fluid. The seeds are mixed with high pressure CO<sub>2</sub> in liquid form (31 °C and 7.3 MPa). Oil dissolves in the CO<sub>2</sub> and when the pressure is released, the CO<sub>2</sub> returns to its gas phase, while the oil precipitates out of the CO<sub>2</sub>-oil mixture. The efficiency of the process depends on die pressure, temperature, contact time and solubility of the oil in CO<sub>2</sub>. Advantages include low temperature, therefore little thermal degradation, shorter extraction time, high selectivity and no solvent residues (Ionescu et al., 2013). Solvents may include compressed gasses such as ethane, propane, ethylene, dinitrogenoxide and CO<sub>2</sub>. The CO<sub>2</sub> at 10–50 MPa and temperature of 35°–80 °C are used. SPE involves heating the solvent to above its critical point at a pressure above 1100 Psi (Nde & Foncha, 2020).

## 2.4 Steam-Distillation (SD)

Steam-distillation (SD) is only used for the extraction of essential oils, which are highly concentrated essences of aromatic plants, and involves the botanical material being placed in a still with steam being forced over the material. The hot steam releases the aromatic molecules from the plant material since the steam forces open the oil-packets in the plant material. These volatile oils escape the plant material and evaporate into the steam. The steam, containing the essential oil, is passed through a cooling system to condense the steam and forms a liquid from which the oil is then separated from the water (Ionescu et al., 2013).

Oils present in plants are used as food (edible oil) and as raw material in the synthesis of polyols, polymers, resins, fuels, soaps, detergents and lubricates in other industries. Oil seeds are natural or “green” reservoirs. Oil extraction methods are categorised by Yusuf (2018) as: (1) Old traditional methods, (2) Conventional methods and (3) Innovative methods, all influencing oil yield, purity and quality.

Generally, seed oil yield depends on the seed variety, soil and environmental conditions (de Wit et al., 2016), as well as the pre-treatment and the extraction method used. Oil yield will be affected by factors such as moisture content of the sample material, particle size and temperature (Yusuf, 2018). Pre-treatment generally includes basic steps such as dehulling, pod or seed coat removal, winnowing, grinding or milling and pre-heating. Oilseeds must be ground or crushed prior to extraction to ensure the minute oil-containing cells that are embedded in fibrous structures, are broken or disrupted to release the oil. Pre-heating, conventionally done by hot-air ovens, is being done by microwave-assisted heat treatments (Yusuf, 2018).

1. Old traditional methods are also referred to as “informal wet extraction methods” used by rural communities. Oils were extracted in three ways: wet extraction (using hot water or steam), solvent extraction and mechanical expression. These methods were described as being crude, largely unscientific, inefficient and yield oils of poor quality (Yusuf, 2018).
2. Conventional methods are well-known and widely used for oil extraction and includes solvent extraction and mechanical expression; sometimes a combination of the two methods may be used (Yusuf, 2018). Solvent extraction is usually applied to oilseeds containing a low (<20%) oil content. It is an efficient method leaving low residual oil in the meal or cake. Solvents normally used include *n*-hexane, diethyl ether, petroleum ether and ethanol (Nde & Foncha, 2020). Soxhlet-based solvent extraction is the primary method used and requires a commercial solvent extractor. This extraction method is effective, produce high yields, is repeatable and reproducible although the cost of solvents is high (Yusuf, 2018).

Mechanical expression involves the application of pressure (hydraulic or screw presses) to force oil out of oil-containing (oleaginous) material. Oil yield is enhanced (increased) by increased mechanical pressure. Screw presses churns out higher yields than hydraulic presses and have a continuous mode of operation. Two types of mechanical press methods exist, i.e. cold-pressed oil

and hot-pressed oil. Cold-pressed, also known as scarification, is done at low temperatures (<50 °C) and pressure, while hot-pressing is done at higher temperatures and pressure. Hot-pressed oils give higher yields because of the decreased oil viscosity at high temperatures, which enhances the flow during extraction. However, the high temperatures might degrade the oil, leading to decreased oxidative stability, degradation of valuable oil components and keeping quality. Cold-pressed oils retains nutraceuticals such as phytosterols and tocopherols (de Wit et al., 2021). Because of these traits, there is a global demand of cold-pressed oils.

3. Innovative techniques, also called unconventional or alternative methods, include microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), supercritical fluid extraction (SFE), Soxtec extraction, microwave-assisted hydro-distillation (MAHD), pressurized liquid extraction (PLE), surfactant-assisted extraction (SAE) and “green” technologies using enzymes [aqueous enzyme extraction (AEE)].

The MAE method is used for isolating vegetable oils from oil seeds as well as extraction of essential oils. Normally pre-treatment of oilseeds is done in a microwave oven that uses radio waves to convey energy and to convert that into heat at a frequency range of 300 MHz–300 GHz. Microwave radiation disrupts the cell membranes of the oilseeds, causing higher extraction yields. MAE results in oils with improved oxidative stability since it allows for better retention of nutraceuticals such as phytosterols, tocopherols, canolol and phenolic compounds. One disadvantage of MAE is that polyunsaturated fatty acids (PUFA) might be degraded resulting in an unrepresentative FA profile (Nde & Foncha, 2020). During MAE, protein material is denatured during microwave treatment leading/causing improved extraction (Nde & Foncha, 2020).

UAE makes use of ultrasonic sound waves to increase vibration and heat. This results in the destruction of the plant cell walls, enhancing the contact of the solvent with the plant material; increasing the oil yield. Ultrasound and ultrasound-assisted extractions use sound waves to produce cavitation microbubbles that collapse in the sample and thereby facilitate the release and extraction of the lipids. Ultrasound-assisted extraction can be done in an open system using a sonicator probe directly on the liquid sample to obtain the seed oil (de Los Angeles Ortega-Ortega et al., 2017). Combinations of innovative techniques are also investigated in an attempt to synergize oil extraction, e.g. ultrasound combined with microwave pre-treatment.

Surfactant-assisted extraction (SAE) uses surfactants as extraction agents with wetting properties, dispersion, solubilisation and emulsification in the method (Djilani & Dicko, 2011). Surfactant concentration and treatment (heating and stirring times) have an influence on the oil yield. Ground seeds are mixed with a surfactant aqueous solution, stirred and heated. Mainly two phases are formed with the upper oil phase collected after centrifugation. Oil yields are similar to that reported for extraction of alkaloids (Yusuf, 2018).



Green technologies such as aqueous enzyme-assisted extraction (AEE) and green solvents such as ionic liquids and terpenes are investigated to replace *n*-hexane as solvent (Mwaurah et al., 2019).

Most conventional techniques, apart from mechanical and hydraulic expression, makes use of solvents in which the oil is dissolved and obtained by evaporation and distillation or by de-emulsification and centrifugation. Green solvents such as water, ethanol, CO<sub>2</sub> and terpenes as well as mixtures of water, ethanol and ethyl acetate could be used as alternatives for *n*-hexane. Some green solvents are non-polar, but have similar properties to conventional solvents and can dissolve similar molecules. “Designer” green solvents include ionic liquids and are non-aqueous solutions of salts prepared by combinations of organic cations and organic or inorganic anions. These can replace organic conventional solvents namely *n*-hexane, petroleum ether, ethyl acetate and chloroform (Mwaurah et al., 2019).

Aqueous enzymatic extraction (AEE) is a novel and green technique using both water and enzymes. In short, enzymes are used to degrade the cell walls of the oil-containing material. Cellulose, hemicellulose and pectin are degraded by enzymes. While the water-soluble portion (the amphipathic lipids) diffuses into the water, the other components form an emulsion. The oil is then de-emulsified either by enzymes or changing the temperature. The major role of the enzymes is to degrade the cell wall of the oilseed to release the oil from the matrix. Enzymes work synergistically, while particle size, pH, temperature, enzyme:substrate ratio, agitation and water:substrate ratio all affect the process. A pre-treatment of the substrate usually assists the effectiveness of the process. These pre-treatments may include ultrasound or high pressure. Microwave-assisted enzymatic extraction (MAEE) is also possible (Mwaurah et al., 2019).

Ultrasound-assisted extraction (USE), also called ultrasonication, is also a green and novel technique. The extraction mechanism involves the production of cavitation bubbles, vibration and pulverization. It involves the disruption of the cell wall, increase the permeability of the cell wall and increase the rate of mass transfer. Ultrasound waves creates a negative pressure in the fluid that results in cavitation, i.e. many tiny bubbles that grow and induce shear forces and turbulence in the liquid as they collapse. The effective frequency ranges to obtain this varies between 20 and 50 KHz (Mwaurah et al., 2019).

Supercritical fluid extraction (SCFE) uses super-critical fluid at the vapour-liquid critical point to extract oil. At the critical point there is no distinctive gas or liquid phase with the solvent behaving more like a gas, but with the solvating properties of a liquid. The super critical state is achieved when the solvent is at a pressure and temperature beyond its critical point. The CO<sub>2</sub> is most commonly used. Characteristics of the sample as well as the interaction between the oil-containing cells and the supercritical CO<sub>2</sub> is of importance. This method is mostly used and effective in extraction of neutral lipids, e.g. triacylglycerides. Ethanol may be added to overcome the problem of low polarity of SC-CO<sub>2</sub> (Mwaurah et al., 2019; Nde & Foncha, 2020). Supercritical fluid extraction (SFE) is sometimes referred to as supercritical

carbon extraction. Other solvents used as co-solvents include methanol, ethanol, isopropanol and acetone. Unlike other methods such as CSE, UAE and MAE, supercritical CO<sub>2</sub> is separated from the extracted oil (Nde & Foncha, 2020). According to Danlami et al. (2014), techniques such as MAE, UAE, PLE and SCFE reduce the disadvantages of the traditional Soxhlet extraction. These methods have shorter extraction times, increased yields, decreased solvent usage and quality improved extracts.

Pulsed electric field-assisted extraction (PEF-AE) is a novel, ground-breaking, non-thermal technology for extraction of vegetable oils from oilseeds. The oleaginous material is placed between two electrodes: a high voltage and a grounded electrode. Direct electric pulses are discharged into the oil-containing material for shorter durations of time ( $\mu\text{s}$  to  $\text{ms}$ ) and at high voltage (to  $\sim 50$  kV). The electric pulses generate electric fields (to  $\sim 10$  kV/cm), which disintegrate membrane molecules. This separation of membrane molecules results in the formation of pores and consequently increase the permeability of the cell wall. The diffusion of solutes is increased by electroporation, enhancing the extraction of oils. PEF pre-treatment on ground and crushed oilseeds increases oil yield. Furthermore, it is also a demulsification technique by facilitating the coalescence of oil molecules in the water/oil emulsion. Thus, because of this double mechanism of electroporation and de-emulsification, the oil recovery and yield is high. It is also classified as a cold technology since temperatures never exceeded 5 °C (Mwaurah et al., 2019).

High pressure-assisted extraction (HPAE) is a novel and rather superior method since it avoids heating of the substrate and therefore preserve bioactive compounds. The process can be divided into high pressure ( $<100$  MPa), medium to high pressure (10–100 MPa), and low pressure ( $<10$  MPa). Regarding the temperature, the process can be divided into pressurized liquid extraction (low temperature) or pressurized hot water extraction (high temperature). High pressure disrupts plant tissue, damage/disrupt the cell walls and membranes, and therefore assist the transfer of soluble material from the substrate to the solvent. This process involves the phase behaviour theory, indicating that solubility of a substance is increased at higher pressure. Thermal degradation of the oil is prevented (Mwaurah et al., 2019).

### 3 Extraction of Active Ingredients

Chemicals known to have medicinal benefits are considered/regarded as “active ingredients” or active principles of natural medicines. Extraction is usually the first step to obtain the natural products from the raw materials according to Zhang et al. (2018a, b). Extraction methods include solvent extraction, distillation, pressing and sublimation. Solvent extraction is the most used method and involves the following processes: (1) solvent penetrates solid matrix, (2) solute dissolves in solvent, (3) solute diffuses out of matrix, and (4) extracted solids are collected. Generally,

extraction is enhanced by finer particle sizes, higher temperatures, longer extraction duration times, and higher solvent-to-solid ratio. Conventional extraction methods include maceration, percolation and reflux extraction, large volumes organic solvents and long extraction times. Maceration could be used for thermos-labile components and could be assisted by microwave- and ultrasound- extraction techniques. Percolation is more efficient than maceration, since it is a continuous process during which the saturated solvent is constantly replaced by fresh solvent. Decoction extraction cannot be used for extraction of thermos-labile or volatile components and contain large amounts of water-soluble impurities. Reflux extraction is more efficient than maceration and percolation and uses less extraction solvent and shorter times. It is also not suitable for the extraction of thermo-labile products.

Soxhlet extraction integrates reflux and percolation extraction, using the principles of reflux and siphoning. It is a continuous method, with high efficiency using high temperature and long extraction times and may cause thermal degradation.

Pressurized liquid extraction (PLE), also described as accelerated solvent extraction, enhanced solvent extraction, pressurized fluid extraction, accelerated fluid extraction and high pressure solvent extraction, uses high pressure in extraction. The high pressure keeps the solvent in a liquid state above its boiling point, resulting in high solubility and high diffusion of lipids into solvent.

Microwaves (Zhang et al. 2018a, b) generate heat by interacting with polar compounds following ionic conduction and dipole rotation mechanisms. The transfer of heat and mass occurs in the same direction during MAE, thus generating a synergistic effect in extraction acceleration and yield. It is regarded as a green technology since it reduces usage of solvents. Two types of MAE exist: solvent free extraction that used for volatile compounds, and solvent extraction that used for non-volatile compounds (Nde & Foncha, 2020). Hydro-distillation (HD) and steam-distillation (SD) are generally used for extraction of volatile oils.

#### **4 Conventional and Non-Conventional Extraction Methods Employed in Cactus Pear Seed Oil Extraction**

Oil contents from oleaginous sources such as oilseeds, nuts, kernels or fruit pulps varies from 3% to 70% of the total weight and extraction thereof are immensely important. Both the oil and the meal (cake) have great economical value. Commercially produced edible oils include soybean, sunflower, nuts (peanuts), rapeseed as well as coconut and palm oil. Non-conventional oils are being embraced because of its multi-purposeness and extraction techniques should be optimized to ensure maximum yields and optimized quality. Extraction is the process of separating triglycerides from oil seeds. This can be done through chemical, biochemical and mechanical techniques already discussed. Extraction methods have moved from conventional solvent and mechanical methods to improved non-conventional

techniques (Nde & Foncha, 2020). Since cactus pear seed oil can be classified as an emerging, non-conventional oil, conventional and non-conventional novel extraction studies done on cactus pear *Opuntia* spp. will be reported.

#### **4.1 Conventional Extraction Methods**

These normally includes the recovery of high added-value compounds from plant matrices using conventional solvents with or without heat treatment. Traditionally, thermal treatments have been used, but heat processing might lead to degradation of heat-sensitive components and might change organoleptic properties (Barba et al., 2017). Typically, plant material (matrices) are homogenized and soaked in a solvent or solvent mixture under constant agitation-molecules of interest are then extracted by diffusion and mass-transfer.

These methods are simple and use inexpensive equipment but have poor efficiency, i.e. recovery and selectivity, high solvent consumption and lower extraction rate. Most industries use conventional methods because of simplicity, ease of maintenance and low capital investment. In the case of seed oils: traditionally used solid-liquid extraction using organic solvents such as *n*-hexane, chloroform, and petroleum ether. Up to 13% seed oil can be extracted, depending on extraction conditions and species.

#### **4.2 Non-Conventional Extraction Methods**

These are non-conventional technologies that employ dedicated processing aids and energy inputs to improve extraction efficiency or selectivity other than that used in conventional extraction methods. These methods include ultrasound (US) micro-waves, supercritical fluids, pulsed electrical fields (PEF) and HVED (high voltage electrical discharges) treatment, as well as mechanical (high pressure processing) HPP and HPH treatments are used to extract valuable compounds from various types of biomasses. It offers superior extraction efficiency in terms of cost yield, time and selectivity. Non-conventional and innovative non-thermal extraction technologies include high pressure processing (HPP), high pressure homogenization (HPH), pulsed electric fields (PEF) and ultra-sound (US) (Barba et al., 2017).

In terms of oil extraction from cactus pear seeds, supercritical CO<sub>2</sub> is a “green” and environmentally safe solvent. Recently, Gharby et al. (2020) used 2-methyloxolane (2-MeO) as a green solvent to replace hexane. It is a bio-based solvent, is produced from renewable raw material and is biodegradable. It is a better extraction solvent

due to the higher polarity than *n*-hexane. Ecocert recognises 2-MeO to produce COSMOS ingredients (Gharby et al., 2020).

## 5 Seed and Oil Contents of *Opuntia* spp.

*Opuntia* seed oil is one of the most valuable plant oils for the food, nutraceutical, pharmaceutical and cosmetic industries. According to Hänke et al. (2018) prices for conventionally produced oil vary between 275 and 700 €/L, and between 900 and 1500 €/L for organic seed oil. It is mainly produced in Morocco, Tunisia and Algeria. The spp. mostly used is *Opuntia ficus-indica*, although spp. such as *O. dillenii* and *O. stricta* are also explored. The PUFA, tocopherols, sterols and phenolics, attributed to *O. ficus-indica* are also present in other *Opuntia* spp. seed oils and as such, provide PUFA (mainly linoleic acid), antioxidants and wrinkle-reducing effects. Concentrations of these compounds may vary. Gharby et al. (2020) reported a most recent market price of 500 €/L.

*Opuntia* seeds contain many health beneficial compounds such as PUFA, phytosterols, sterols, fat-soluble vitamins e.g. vitamin E (tocopherols) and  $\beta$ -carotene (Koubaa et al., 2016; de Wit et al., 2021). Oils are usually extracted and obtained from the seeds, although the pulp also contains oils (Ramadan & Mörsel, 2003a, b). Oil contents vary between species and cultivars, different fruit colours as well as because of the effect of locations, agricultural conditions (seasons, rainfall, and temperature) and fruit maturity stage. Typically, the seed oils are rich in linoleic acid (polyunsaturated *omega*-6) fatty acids (de Wit et al., 2016, 2017, 2018, 2021). Other authors (Ramírez-Moreno et al., 2017; Brahmi et al., 2020) also specify genetic factors, cultivars, growth conditions, harvesting routine, degree of ripeness, fruit handling and storage, soil characteristics, geographical fluctuations, crop environmental factors (light, temperature, soil nutrients) as well as chemical compounds present in the source influencing oil yield and quality.

## 6 Extraction of the Oils from *Opuntia* spp.

Extraction method has a definite effect on the oil yield. The progression in extraction methods used, as well as the effect of species, cultivar, location (country) of different research studies on cactus pear seed oils are indicated in Tables 25.1 and 25.2. A distinction is made between conventional (Table 25.1) and non-conventional (Table 25.2) extraction methods.

**Table 25.1** Conventional oil extraction methods

Specie	Extraction method	Oil yield	Country	Reference
<i>O. ficus-indica</i>	ND	4.6–17.2%		Sawaya and Khan (1982), Sawaya et al. (1983)
<i>O. ficus-indica</i>	Hexane	8–9% (9.14%)	Italy	Salvo et al. (2002)
<i>O. ficus-indica</i>	Methanol/ chloroform	9.9%	Germany	Ramadan and Mörseel (2003a, b)
<i>O. ficus-indica</i>	ND	6.91% (up to 14%)	Turkey, Morocco	Coşkuner and Tekin (2003)
<i>O. stricta</i>	ND	11.05%	Tunisia	Ennouri et al. (2005)
	Hexane			El-Mannoubi et al. (2009)
<i>O. dillenii</i>	Petroleum ether/ chloroform Soxhlet	6.01%	China	Liu et al. (2009)
<i>O. ficus-indica</i>	Chloroform/ methanol Soxhlet	2.24–5.69%	South Africa	Labuschagné and Hugo (2010)
<i>O. ficus-indica</i>	ND	5%	Turkey	Özcan and Al Juhaimi (2011)
<i>O. ficus-indica</i>		9.88– 11.75% 5–14%	Tunisia Turkey	Matthäus and Özcan (2011)
<i>O. ficus-indica</i>	Petroleum ether	4.4%; 5.41% & 6.85%	Tunisia	Tlili et al. (2011)
<i>O. joconostle</i>	Soxhlet	2.45%	Mexico	Morales et al. (2012)
<i>O. matudae</i>	ND	3.52%	Mexico	Morales et al. (2012)
<i>O. ficus-indica</i>	Hexane maceration		Morocco	Ghazi et al. (2013)
<i>O. dillenii</i>	Hexane maceration		Morocco	Ghazi et al. (2013)
<i>O. elatior</i>	Petroleum ether Soxhlet			Bhatt and Nagar (2013)
<i>O. ficus-indica</i>	Hexane Soxhlet	7.3–9.3%	Algeria	Chougui et al. (2013)
<i>O. macrorrhiza</i> (Engelman)	ND	9.2%	Tunisia	Chahdoura et al. (2015)
<i>O. microdasys</i>	ND	11.3%	Tunisia	Chahdoura et al. (2015)
<i>O. stricta</i>	Hexane Soxhlet	49%		Koubaa et al. (2016)
<i>O. ficus-indica</i>	Chloroform/ methanol Soxhlet	5.66–7.59	South Africa	De Wit et al. (2016)
<i>O. ficus-indica</i>	Chloroform/ methanol Soxhlet	5.65–8.09%	South Africa	De Wit et al. (2017)
<i>O. robusta</i>	Chloroform/ methanol Soxhlet	5.65–8.09%	South Africa	De Wit et al. (2017)
<i>O. ficus-indica</i>	Hexane Soxhlet	>7%		de Los Angeles Ortega-Ortega et al. (2017)

(continued)

**Table 25.1** (continued)

Specie	Extraction method	Oil yield	Country	Reference
<i>O. ficus-indica</i>	Hexane Maceration	>5%		de Los Angeles Ortega-Ortega et al. (2017)
<i>O. ficus-indica</i>	Hexane, ethanol, ethyl acetate	6.89%	Mexico	Ramírez-Moreno et al. (2017)
<i>O. albicarpa</i>	Hexane, ethanol, ethyl acetate	11.83%	Mexico	Ramírez-Moreno et al. (2017)
<i>O. ficus-indica</i>	Cold-pressed		Sicily, Italy	Ciriminna et al. (2017)
<i>O. ficus-indica</i>	Soxhlet	9.3–9.5%	Sicily, Italy	Loizzo et al. (2019)
<i>O. ficus-indica</i>	Chloroform/methanol Soxhlet	4.09–8.76%	South Africa	De Wit et al. (2018)
<i>O. robusta</i>	Chloroform/methanol Soxhlet	5.23–5.65	South Africa	De Wit et al. (2018)
<i>O. ficus-indica</i>	Soxhlet	6.42%	Algeria	Farah et al. (2018)
<i>O. albicarpa</i>	Maceration-percolation n-hexane	8.72%	Sicily, Italy	Regalado-Rentería et al. (2018)
<i>O. megacantha</i>	Maceration-percolation n-hexane	7.63	Sicily, Italy	Regalado-Rentería et al. (2018)
<i>O. matudae</i>	Maceration-percolation n-hexane	9.68	Sicily, Italy	Regalado-Rentería et al. (2018)
<i>O. streptacantha</i>	Maceration-percolation n-hexane	10.55–11.64	Sicily, Italy	Regalado-Rentería et al. (2018)
<i>O. robusta</i>	Maceration-percolation n-hexane	14.54–15.54	Sicily, Italy	Regalado-Rentería et al. (2018)
<i>O. albicarpa</i>	Cold-pressed (hydraulic)	1.19%	Sicily, Italy	Regalado-Rentería et al. (2018)
<i>O. megacantha</i>	Cold-pressed (hydraulic)	1.66	Sicily, Italy	Regalado-Rentería et al. (2018)
<i>O. matudae</i>	Cold-pressed (hydraulic)	1.65	Sicily, Italy	Regalado-Rentería et al. (2018)
<i>O. streptacantha</i>	Cold-pressed (hydraulic)	0.51–2.52	Sicily, Italy	Regalado-Rentería et al. (2018)
<i>O. robusta</i>	Cold-pressed (hydraulic)	5.71–6.08	Sicily, Italy	Regalado-Rentería et al. (2018)
<i>O. ficus-indica</i>	Cold pressure			Khémiri et al. (2019)
<i>O. ficus-barbarica</i>	Petroleum ether Soxhlet	6.8–9.81%		Al Juhaimi et al. (2020)
<i>O. ficus-indica</i>	Cold-pressed		Algeria	Brahmi et al. (2020)
<i>O. ficus-indica</i>	n-hexane Soxhlet	5.4%	Greece	Karabagias et al. (2020)
<i>O. ficus-indica</i>	Hexane Soxhlet	88 g/kg	Saudi-Arabia	Koshak et al. (2020)

(continued)

**Table 25.1** (continued)

Specie	Extraction method	Oil yield	Country	Reference
<i>O. ficus-indica</i>	n-hexane	8.86	Marocco	Gharby et al. (2020)
<i>O. dillenii</i>	Petroleum ether	7.04	Madagascar	Hänke et al. (2018)
<i>O. stricta</i>	Petroleum ether	8.8	Madagascar	Hänke et al. (2018)
<i>O. ficus-indica</i>	Cold-pressed	2.51–5.96%	South Africa	De Wit et al. (2021)

ND not described

**Table 25.2** Non-conventional extraction methods

Specie	Extraction Method	Oil yield	Country	Reference
<i>O. ficus-indica</i>	Supercritical CO <sub>2</sub>		China	Liu et al. (2009)
<i>O. ficus-indica</i> spiny	Supercritical CO <sub>2</sub> and Soxhlet	3.4%		Yeddes et al. (2012)
<i>O. ficus-indica</i> spineless	Supercritical CO <sub>2</sub> and Soxhlet	1.94%		Yeddes et al. (2012)
<i>O. ficus-indica</i>	Hydro-distillation n-hexane	9.55	Sicily	Zito et al. (2013)
<i>O. stricta</i>	SC-CO <sub>2</sub>	49.9%		Koubaa et al. (2016)
<i>O. ficus-indica</i>	Ultrasound and ultrasound-assisted	3.75–6%		de Los Angeles Ortega-Ortega et al. (2017)
<i>O. ficus-indica</i>	Ultrasound-assisted	5.4–5.6	Sicily	Loizzo et al. (2019)
<i>O. ficus-indica</i>	Hydro-distillation	5–14.4%	Turkey	Ali Alsaad et al. (2019)
<i>O. dillenii</i>	Hydro-distillation	6.5%	Iraq	Ali Alsaad et al. (2019)
<i>O. dillenii</i>	Supercritical CO <sub>2</sub>	6.65%		Liu et al. (2009)
<i>O. ficus-indica</i>	2-MeO		Marocco	Gharby et al. (2020)

## 7 Conclusion

Cactus pear seed oils are of great economic importance for not only the producers, but also the food, pharmaceutical, health and cosmetic industries. The oils are excellent sources of bioactive compounds including essential fatty acids (*omega-6* linoleic acid), vitamins, tocopherols and sterols. Conventional and non-conventional extraction methods are employed to extract the oil from the seeds. Cold-pressing and solvent extraction methods is still mostly used. All extraction methods have their own advantages and drawbacks. Extraction conditions will influence the oil yields as well as the extraction efficiency of the various bioactive compounds. In summary, valuable products can be obtained from waste products contributing to adding value-added revenue for arid and semi-arid regions of the world.



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## Chapter 26

# Novel Pectins from Prickly Pear (*Opuntia albicarpa*) Fruits: Structural Features and Rheological Properties



Adriana Inés Rodríguez-Hernández and Norberto Chavarría-Hernández

**Abstract** Pectins are structurally heterogeneous polysaccharides present in higher plant cell walls. The structural complexity and macromolecular properties of pectins are dependent on their source and the extraction method used. In the last decades, there has been a tremendous scientific interest in pectins, which is accounted for the broad spectrum of their techno-functional properties (gelling, thickening, stabilizing, and film-forming) and health-benefit effects (prebiotic, antioxidant, and anticancer). These properties are strongly related to their unique structure. Commercial pectins are mainly isolated from citrus peel and apple pomace; however, the studies concerning novel pectin sources attract growing scientific attention. This chapter describes the extraction process and the main chemical, macromolecular and rheological properties of novel pectin obtained from the peel of cactus pear, *Opuntia albicarpa*, a well-valued fruit by its taste, in Mexico. The pectin from *Opuntia albicarpa* is a high-molecular-weight polysaccharide, characterized as acetylated-low methoxyl pectin. Its solutions show non-Newtonian rheological behavior and gelling ability in the presence of calcium ions. These unique features offer the possibility of its use as a new natural polysaccharide for diverse applications such as food, personal care products, nanomaterials in drug delivery, and biomaterial for packaging uses.

**Keywords** Techno-functional properties · Prickly pear · Pectic polysaccharide · Rheological properties · Acetylated pectin

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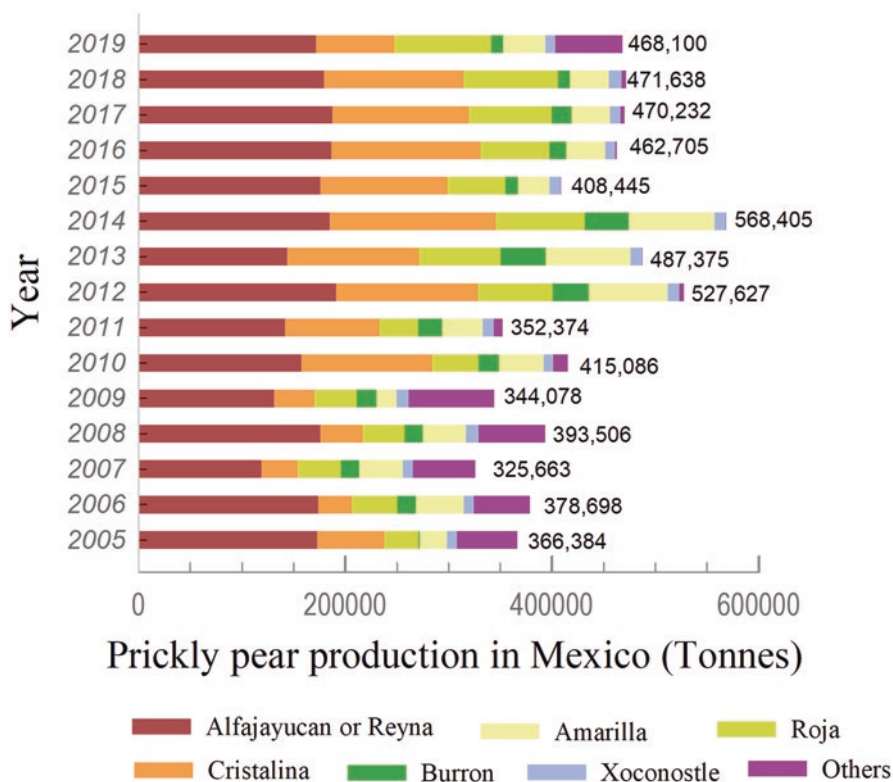
## 1 Fruits of *Opuntia*

*Opuntia* is a diverse genus of the Cactaceae family, including 188 species (Anderson, 2001). *Opuntia*'s domestication process is believed to have occurred in Mexico; the country is considered the most important center of *Opuntia*'s diversity. A total of 83 *Opuntia* species have been reported in Mexico (Chávez-Moreno et al., 2009), where nearly half of them are endemic (Anderson, 2001). During the humanization process of *Opuntia*, the continuous and systematic gathering of “nopalitos” (edible young cladodes) and fruits (prickly pears) promoted the development of exceptional features. For the case of prickly pears, this process enhanced their flavor, size, shape, pulp texture, and decreased the seed hardness and seed quantity (López-Palacios et al., 2011). Thus, there is an extraordinary wealth of *Opuntia* variants with different degrees of domestication in Mexico, from those harvested or planted as living fences and edges of slopes of plots and those of solar, even of commercial plantations.

The main species grown in Mexico are *O. albicarpa*, *O. megacantha*, *O. streptacantha*, *O. ficus-indica*, *O. robusta*, *O. hyptiacantha*, and *O. joconostle*, with more than 102 cultivars highly appreciated by their fruits. The Mexican Ministry of Agriculture, Livestock, Rural Development, Fishing and Food (SAGARPA), through the Food, Agriculture and Fishing Information Service (SIAP), records the main varieties of prickly pears produced in Mexico: Alfajayucan or Reyna (*O. albicarpa* Scheinvar), Blanca Cristalina (*O. albicarpa* Scheinvar), Amarilla (*O. megacantha*), Roja (*O. ficus-indica* (L.) Mill.), Blanca Burrón (*O. albicarpa*), and Xoconostle (*O. joconostle*; *O. matudae*). Figure 26.1 shows the information for the production of those prickly pears in the 2005–2019 period (SIAP, 2020), which has remained at about 470,000 t/year in the last 4 years. Alfajayucan and Blanca Cristalina are the two prickly pear cultivars most cultivated and commercialized in Mexico; in 2019, both cultivars represented 53% of total prickly pears harvested on a surface of 24,722 ha. Even though *Opuntia* fruits are also grown in other countries, including India, United States of America, South Africa, Chile, Italy, and Argentina, Mexico is the world leader producer. This fruit is ancestrally crucial in Mexico, and the geographic and climate characteristics of the country allow its cultivation. Barba et al. (2017) reported that 44% of the World's prickly pears production is carried out in Mexico.

## 2 *Opuntia albicarpa* Scheinvar (‘Reyna’)

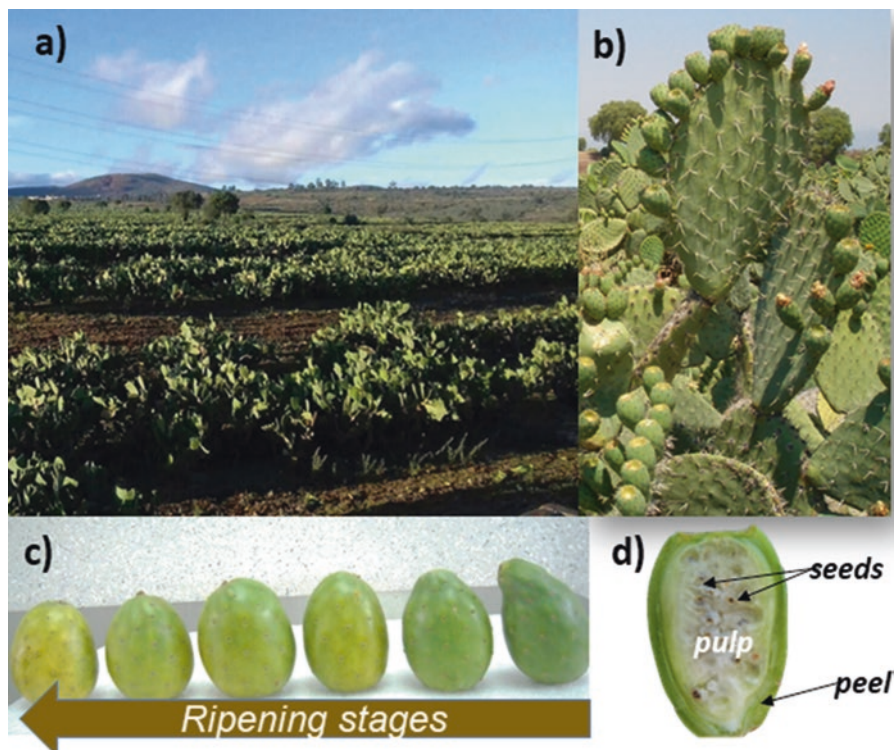
*Opuntia albicarpa* Scheinvar cv. Reyna, also known as ‘Alfajayucan’ cultivar, is the prickly pear most cultivated in Mexico, mainly in the center of the country (Estado de México and Hidalgo). Figure 26.2 shows the main characteristics of the nopal plant and the fruits of *Opuntia albicarpa* Scheinvar cv. Reyna. The harvest season of this cultivar is July and August. The prickly pear is a fruit with an obovate shape, 6–9 cm in length. Its peel is thin (5–7 mm) with a low number of areoles per fruit



**Fig. 26.1** Production of the main cultivars of prickly pears (*Opuntia* spp.) in Mexico during the period 2005–2019. Source: The Mexican Ministry of Agriculture, Livestock, Rural Development, Fishing and Food (SAGARPA)

and distant from each other, and its color ranges from yellowish-green (ripe fruit) to dark green (unripe) (Fig. 26.2c). Its pulp is whitish-green, sweet ( $\approx 15^\circ\text{Bx}$ ), juicy, and plentiful of non-edible seeds (Fig. 26.2d) (Scheinvar, 1999).

Table 26.1 shows the physical characteristics of *Opuntia albicarpa* Scheinvar cv. Reyna from San Martín de las Pirámides, Estado de México, Mexico ( $19^\circ 66'98''$   $72'$ ). The thinness of this cultivar's peel is a highly valued characteristic for its consumption but limits its shelf life, making it more susceptible to mechanical damage. The peel comprises around 40% of the fresh fruit weight; since it is not edible, it is considered an agro-industrial waste, an alternative source of high added-value products such as polysaccharides (pectins, mucilages, and insoluble dietary fiber) and phenolic compounds. Although most studies have focused on the extraction of bioactive compounds from cladodes and prickly pear peel from *Opuntia* spp., mainly *O. ficus-indica* (Amaya-Cruz et al., 2019; Angulo-Bejarano et al., 2014; Bensadón et al., 2010; Gheribi et al., 2019; Melgar et al., 2017), only a few studies have been reported on the extraction of bioactive compounds from *Opuntia albicarpa* (Lira-Ortiz et al., 2014; López-Palacios et al., 2016; Morales-Martínez et al., 2018).



**Fig. 26.2** *Opuntia albicarpa*: (a) A plantation in San Martín de las Pirámides, Estado de México, Mexico, (b) close view of the nopal plant and its fruit (prickly pear), (c) fruits at different ripening stages, and (d) transversal view of a prickly pear at physiological maturity

**Table 26.1** Physical characteristics of prickly pear (*Opuntia albicarpa* Scheinvar cv. Reyna) fruits

Feature	Mean	Standard deviation
Length (mm)	78.91	6.41
Larger diameter (mm)	52.71	2.91
Length/larger diameter ratio	1.50	0.11
Peel thickness (mm)	4.47	1.03
Fruit weight (g)	112.62	15.91
Pulp weight (g)	67.59	10.91
Peel weight (g)	44.21	8.31
Peel weight/pulp weight, ratio	0.66	0.14
Total solids in peel (% w/w)	9.54	0.62
Total soluble solids in the pulp (% w/w)	15.00	0.5

Mean values ( $n = 60$ ) and standard deviation



Therefore, it is imperative to develop technological platforms to obtain bioactive compounds with high potential applications such as food additives, functional ingredients, personal care products, biomaterials, and the sustainable use of prickly pears.

### 3 Pectins from *Opuntia* spp.

Nowadays, there is a growing interest in natural and safe ingredients worldwide. The bio-based polymers research is increasing mainly to examine novel sources of biopolymers or improve their techno-functional properties for diverse biotechnological applications. Pectin is the term that describes a family of complex structural polysaccharides contained in the cell walls of plants. It was discovered in 1825 by Henri Braconnot while he examined the gel-forming substances isolated from apple juice (Ciriminna et al., 2015). Almost two centuries later of its discovery, studies about pectins' functional and bioactive properties are on the rise. The traditional food applications of pectins are widely known; they have been used as soluble dietary fiber or thickening, gelling, and stabilizing agents. However, other uses of pectin and its derived products have been investigated in the last decades. Many studies evidence bioactive activities or health effects associated with pectin and modified pectin. They include the cholesterol-lowering, serum glucose-lowering, anti-inflammatory, hypoglycemic, antibacterial, antioxidant, and anticancer activities (Minzanova et al., 2018; Naqash et al., 2017). Additionally, new research is carried out to assess the use of several pectins in drug delivery, wound healing, tissue engineering, and bio-based packaging (Calce et al., 2014; Minzanova et al., 2018; Rivera-Hernández et al., 2020).

The chemical features of pectins determine their bioactive and functional activities. Pectin is considered as one of the most complex macromolecules in nature. It is a heteropolysaccharide containing galacturonic acid units predominantly, in which some of these units may be methyl-esterified or acetylated. Most scholars coincide with pectin-structural elements, namely homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II, arabinogalactan I, arabinogalactan II, arabinan, and xylogalacturonan. It is believed that all pectins contain the same structural elements, but the amount and chemical structure among them can differ, resulting in the complexity and structural diversity of the pectins (Voragen et al., 2009).

Thus far, commercial pectins are obtained from citrus peel, apple pomace, and sugar beet all over the World. Nevertheless, numerous research groups have reported pectins extracted from diverse sources, a lot of them from vegetable wastes such as cacao pod husks (Vriesmann & de Oliveira Petkowicz, 2017), sisal waste (Santos et al., 2013), pomace grape -winery- (González-Centeno et al., 2010) and peel of various fruits (i.e., banana, dragon fruit, grapefruit, pomegranate, mango, jackfruit, melon, papaya, passion fruit, and prickly pears among others) (do Nascimento Oliveira et al., 2018; Kalegowda et al., 2017; Lira-Ortiz et al., 2014; Marena et al., 2019; Morales-Martínez et al., 2018; Zhuang et al., 2019).

*Opuntia* plants have been explored as a source of polysaccharides since ancestral times, cladodes have been used to obtain mucilages and pectins, and to a lesser extent, some *Opuntia* fruits have been assessed as a source of pectins. Table 26.2 summarizes the main findings of studies on the extraction and characterization of pectic polysaccharides from *Opuntia*'s fruits. Few *Opuntia* species have been evaluated as a source of pectins, being *O. ficus-indica*, the most reported. The extraction yields ranged from 7.3% to 18.2%, which are lower than those reported for industrial pectins from the citrus peel (15–30%) and close to commercial pectins from apple pomace (10–15%) and other pectins from non-conventional sources as sugar beet (4.1–16.2%) or passion fruit ( $\approx 8\%$ ). The conventional extraction processes use mineral or organic acids (pH 1–3), high temperatures (60–100 °C), and often large processing times (Freitas de Oliveira et al., 2016; Marić et al., 2018; Yapo & Gnakri, 2015; Yapo et al., 2007). In recent years, there has been a global trend toward assessing emerging processing techniques (microwave, enzymatic, and ultrasound-assisted extraction) to obtain pectin from other wastes and by-products. Most studies showed that microwave-assisted extraction and ultrasound-assisted extraction often increase the yield extraction, reduce the time and energy consume (Marenda et al., 2019). However, these innovative processing techniques need to be optimized and standardized according to the plant material because the chemical structure and, thus, pectins' functional properties are strongly influenced by the extraction conditions.

#### 4 Prickly Pears of *Opuntia albicarpa*: A Novel Source of Pectins

Since several years ago, our research group has investigated the extraction and characterization of pectin polysaccharides from prickly pears of *O. albicarpa*, motivated by the limited technological developments implemented to utilize the by-products of this prickly pear, very marketable as fresh fruit in central Mexico. The pectin extraction protocol was previously described (Lira-Ortiz et al., 2014), and it is summarized in Fig. 26.3. Fresh prickly pear fruits (*O. albicarpa* Scheinvar) from San Martín de las Pirámides, Estado de México, Mexico (19° 66'98" 72') were harvested at physiological maturity. The peel was removed from fruits and then reduced by grinding for a few minutes in a domestic food processor. The vegetal material was steam-blanching for 20 min for enzymatic inactivation, and after, it was subjected to a two-stage extraction into a jacketed glass tank (3 L processing volume) with constant stirring. Briefly, (1) peel leaching with deionized water for two h at 60 °C; (2) the supernatant was discarded, and the wet peels were leached again with an aqueous solution of 1 wt% ethylenediaminetetraacetic acid (pH = 4.0) for two h at 70 °C. The solid/solvent ratio used in the extraction process was 300 g/L. The polysaccharides extracted in the second stage (calcium-bound pectins) were precipitated using 96% acidified ethanol. After that, the polysaccharide was washed

**Table 26.2** Physico-chemical characterization of pectic polysaccharides from *Opuntia* spp.

Source	Extraction yield (%) <sup>a</sup>	GalA (%) w/w)	DE (%)	Mw (g/mol)	Neutral sugar (% w/w) <sup>b</sup>							Reference
					Glu	Xyl	Gal	Rha	Ara	Man		
<i>O. ficus-indica</i>	0.12 <sup>c</sup>	64.3	20	–	2.5	2.1	22.2 <sup>d</sup>	–	5.8	–	Forni et al. (1994)	
<i>O. ficus-indica</i>	7.3	51.8	32	–	0	0	48.2	0	–	–	Majdoub et al. (2001)	
<i>O. ficus-indica</i>	12.4	64.5 <sup>d</sup>	–	–	–	0.9	4.0	3.7	17.2	–	Habibi et al. (2004)	
<i>O. albicarpa</i>	9.8 ± 0.6	65.4	30	–	0.12	ND	19.5	2.16	0.12	ND	Lira-Ortiz et al. (2014)	
<i>O. dillenii</i>	16.3–18.2	66.8–74.0	63–85	21,700–76,200	2.9–6.6	0.2–1.9	10.2–12.4	0.9–3.2	4.4–5.4	0.4–7.0	Kalegowda et al. (2017)	
<i>O. albicarpa</i>	13.66 ± 2.00	65.2 ± 0.9	43	204,080	–	–	–	–	–	–	Morales-Martínez et al. (2018)	
<i>O. matudae</i>	10.46 ± 2.560	75.7 ± 8.2	50	161,730	–	–	–	–	–	–	Morales-Martínez et al. (2018)	
<i>O. robusta</i>	15.7	47.1	<50	20,340	–	–	–	–	–	–	Mota et al. (2020)	

*GalA* galacturonic acid, *Glu* glucose, *Xyl* xylose, *Gal* galactose, *Rha* rhamnose, *Ara* arabinose, *Man* mannose, *ND* not detected

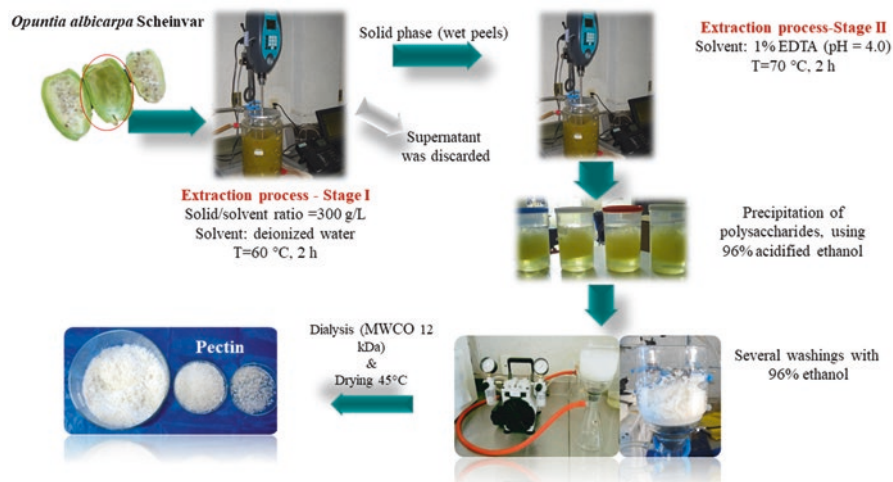
<sup>a</sup>Referred to the dry weight of prickly pear peel

<sup>b</sup>Referred to the dry weight of extract, expressed in relative weight percentages

<sup>c</sup>Referred to the wet weight of prickly pear peel

<sup>d</sup>Reported as uronic acids

<sup>e</sup>As Gal + Rha



**Fig. 26.3** Experimental protocol used to extract and purify pectins from fruits of *Opuntia albicarpa* Scheinvar ‘Reyna’

**Table 26.3** Physico-chemical characterization of pectin from peels of *Opuntia albicarpa* cv. Reyna

Extraction yield (g <sub>AIS</sub> <sup>a</sup> /100 g dry vegetal material)	10.32 ± 0.95
Galacturonic acid (% w/w, db)	69.5 ± 2.30
Arabinose	7.7
Galactose	5.7
Rhamnose	1.8
Glucose	0.6
Mannose	0.12
Protein (% w/w, db)	4.4 ± 0.30
Esterification degree (DE, %)	43.80 ± 2.10
Degree of methyl esterification (DM, % mol)	44.60 ± 0.50
Degree of acetylation (DA, % mol)	10.60 ± 0.20
Weight-average molecular weight (M <sub>w</sub> , g mol <sup>-1</sup> )	240,100

<sup>a</sup>AIS alcohol insoluble solids, db dry basis. Data are means ± standard deviations; n = 3

successively with 96% ethanol. Finally, the polysaccharide was dried at 45 °C, ground, and sieve to produce a homogeneous powder stored for later characterization.

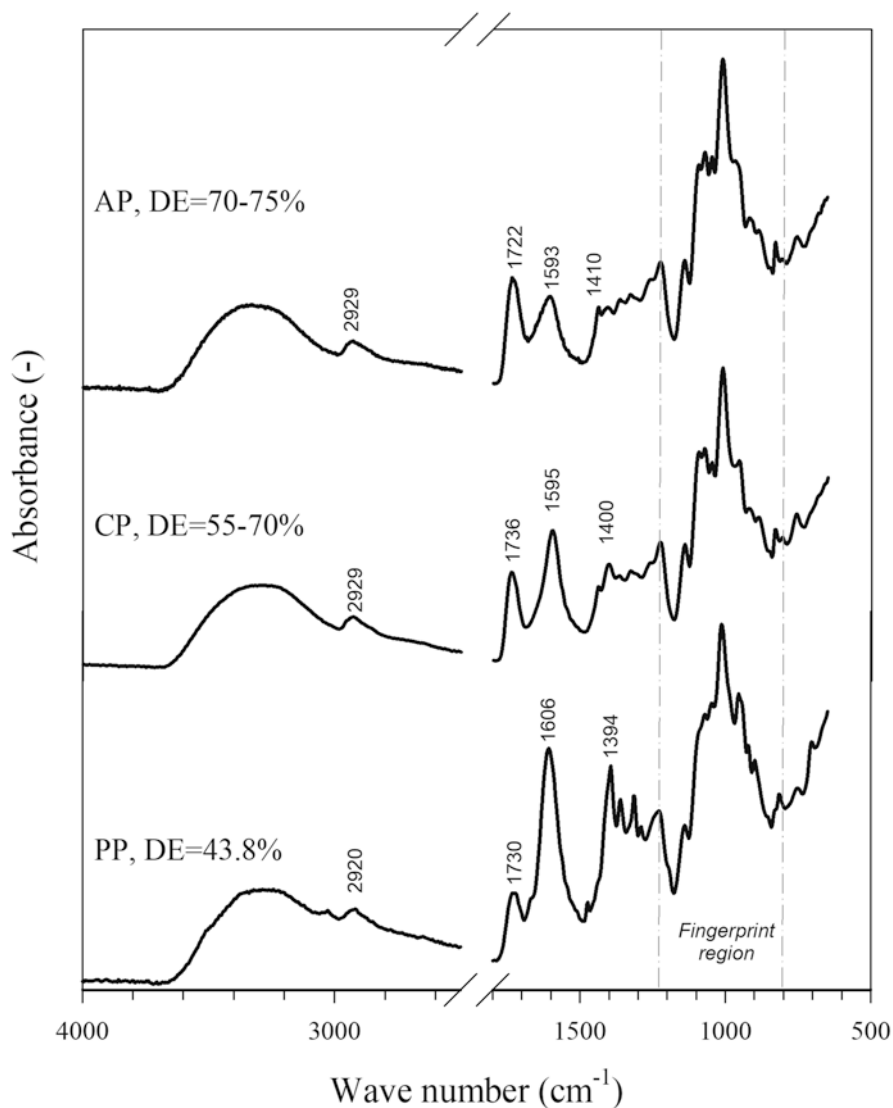
The yield of extraction of pectins from prickly pears of *O. albicarpa* and the main physico-chemical properties are summarized in Table 26.3. The yield of extraction was 10.3 g/100 g dry matter. This value is reasonably similar to those

previously reported for pectins from *Opuntia* fruits (see Table 26.2). The variations may be due to differences in species and cultivars, the stage of maturity of fruits, and the downstream operations. The content of galacturonic acid (GalA) is an essential parameter for recognizing a pectic polysaccharide as 'pectin' for food or cosmetics uses. FAO and the European Union stipulate that 'pectin' must consist of at least 65% GalA (Rolin, 2002; Willats et al., 2006). Thus, according to the GalA content (69.5%), polysaccharides from *O. albicarpa* can be recognized as 'pectin.' Previous works about pectins from *O. albicarpa* have reported GalA contents close to 65%, using the same extraction protocol (Lira-Ortiz et al., 2014; Morales-Martínez et al., 2018).

On the other hand, the composition of the neutral sugars is sensitive to extraction and downstream conditions. Monosaccharide units' composition varies from molecule to molecule in any pectin sample (Dranca & Oroian, 2018). Arabinose, galactose, and rhamnose are the main constituent sugars in pectins from prickly pears of *O. albicarpa*, which suggest that arabinogalactans can be part of the branched regions of long chains of homogalacturonan. That is in agreement with the low content of rhamnose and the high average molecular weight ( $M_w = 240,100 \text{ g mol}^{-1}$ ) of the pectin.

The functional properties of pectins are determined mainly by the number of non-esterified GalA units and their distribution along the homogalacturonan chain and the distribution of molecular weights. Acetylation and methylation of pectins also substantially affect their gelling, surface tension, and emulsification properties. According to the chemical composition and macromolecular properties (Table 26.3), the pectin obtained from peels of prickly pears of *O. albicarpa* is acetylated-low methoxyl pectin with significant protein content (4.4%) and high enough molecular weight ( $240,100 \text{ g mol}^{-1}$ ), which have a positive effect on the gel formation ability and thickening properties as it is discussed later.

Fourier transform infrared (FT-IR) spectroscopy was used to identify the main functional groups of pectin macromolecule. Figure 26.4 shows the FT-IR spectra of the pectins from prickly pears of *Opuntia albicarpa* (PP), citrus pectin (CP, Sigma-Aldrich P9436; DE = 55–70%), and apple pectin (AP; Fluka Analytical DE = 70–74%), the later ones used as reference pectins. The inspection of the spectra allows identifying similarities among the absorbance patterns of the three pectins. A broad and intense band was evident between  $3000$  and  $3700 \text{ cm}^{-1}$ , assigned to O-H stretching of bound water. Smaller bands near  $2900$  can be attributed to the C-H stretching vibration of the  $\text{CH}_3$  groups of methyl and acetyl esters (Synytsya et al., 2003). The functional group region between  $1500$  and  $1800 \text{ cm}^{-1}$  is fundamental to determine the degree of esterification (DE) of pectins. The  $1730 \text{ cm}^{-1}$  band in the PP spectrum ( $1736$  and  $1722$  for CP and AP, respectively) is assigned to C=O stretching vibrations of the non-ionized or esterified carboxylic groups; the bands at  $1593$ – $1606 \text{ cm}^{-1}$  correspond to the absorption of the carboxylate anions ( $\text{COO}^-$ ). It has been reported that the absorbance intensity of the  $1720$ – $1750 \text{ cm}^{-1}$  bands (esterified carboxylic groups) increases with the increase in the DE value of the pectins, whereas a decrease in the absorbance intensity of the bands near  $1600 \text{ cm}^{-1}$  (carboxylate stretching) is observed (Chatjigakis et al., 1998).



**Fig. 26.4** FTIR spectra for: apple pectin (AP; Fluka Analytical DE = 70–74%), citrus pectin (CP, Sigma-Aldrich P9436; DE = 55–70%), and pectin from prickly pear peel of *Opuntia albicarpa* Scheinvar ‘Reyna’

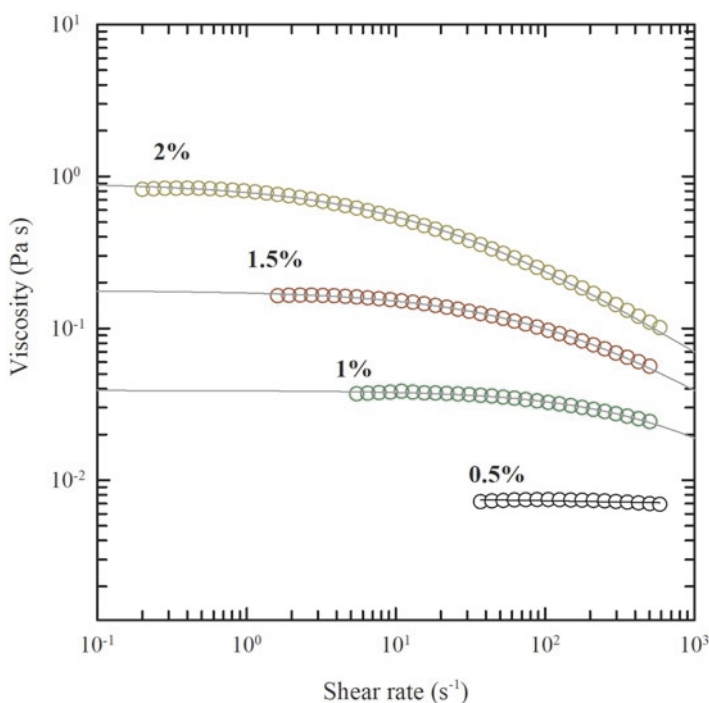
Accordingly, the relative intensity of those bands in the FT-IR spectra of PP, CP, and AP corroborates their respective DE values of the pectin samples. The band near  $1400\text{ cm}^{-1}$  confirms carboxylate anions, which is more intense in the PP spectrum. The region between  $800$  and  $1200\text{ cm}^{-1}$ , known as the ‘fingerprinter region,’ is dominated by ring vibrations overlapped with C-OH side groups’ stretching vibrations

and the C-O-C glycosidic bond vibration. In this region, the absorption bands cannot unambiguously be assigned to any particular vibration (Chatjigakis et al., 1998); nonetheless, we can corroborate the similarities based on the similarities pectic nature of PP and its identity as low-methoxyl pectin.

## 5 Rheological Properties of Pectins from Prickly Pears of *Opuntia albicarpa*

### 5.1 Flow Behavior

Figure 26.5 shows the rheological flow properties of aqueous pectin solutions at 25 °C. The viscosities of pectin solutions increase with pectin concentration, a typical flow behavior of hydrocolloids in an aqueous solution. As the pectin concentration increases, the pectin molecules' intermolecular physical interactions are promoted, such as hydrogen bonding and hydrophobic interactions, leading to more resistance to flow. All pectin solutions behave like Non-Newtonian fluids; the



**Fig. 26.5** Viscosity curves of aqueous solutions of prickly pear pectin from *Opuntia albicarpa* Scheinvar 'Reyna'. The lines represent the best fit to Cross equation. Tests carried out at 25 °C in an ARES-G2 rheometer (TA Instruments, USA) using the cone and plate fixture (40 mm, 2°)

**Table 26.4** Cross-equation and Ostwald-de Waele model parameters for aqueous solutions of pectin from prickly pear peels

Pectin concentration (%)	Cross equation				
	$\eta = \frac{\eta_0}{1 + (\lambda \dot{\gamma})^{1-n}}$				
	$\eta_0$ (mPa s)	$\lambda$ (s)	$n$ (-)	$R^2$	Standard error of estimate
2	909	0.054	0.38	0.998	0.0103
1.5	177	0.007	0.35	0.998	0.0015
1.0	39	0.001	0.25	0.992	0.0004
	<i>Ostwald-De Waele equation</i>				
	$\eta = K \dot{\gamma}^{(n-1)}$				
	$K$ (mPa s <sup>n</sup> )	$n$ (-)		$R^2$	Standard error of estimate
0.5	9	0.96		0.999	0.0153

viscosity was dependent on shear rate, decreasing as the shear rate increased. This shear-thinning behavior, also known as pseudo-plasticity, was magnified at pectin concentrations higher than 1.0% w/w. A Newtonian plateau can describe the pectin solution's viscosity curves at low shear rates, followed by a shear thinning behavior. The three-parameters Cross equation can well describe the flow behavior of pectin systems depicted in Fig. 26.5 (see Table 26.4), extensively used to explain the rheological flow behavior of polysaccharide systems where the upper Newtonian plateau ( $\eta_\infty$ ) is often experimentally inaccessible, and the exponent can be represented as “ $1-n$ ”, being  $n$  the power-law flow behavior index (Rao, 2014).

Table 26.4 presents the rheological parameters for pectins from prickly pears of *Opuntia albicarpa*. Experimental data of 1–2% pectin solutions fitted well to the Cross equation, as illustrated by the regression coefficient ( $R^2$ ) and the standard error of estimates. Otherwise, the flow behavior of 0.5% pectin was described by the Ostwald-de Waele model as previously reported (Lira-Ortiz et al., 2014). The zero shear Newtonian region, characterized by  $\eta_0$  values, increased as the pectin concentration did, suggesting an enhancing of the macromolecular entanglements as polymer concentration increases, which are then disrupted at higher shear rates. The Cross time parameter  $\lambda = 1/\dot{\gamma}_c$  defines the critical shear rate ( $\dot{\gamma}_c$ ) or the onset of the shear-thinning region (Rao, 2014). The rheological flow behavior of prickly pear pectins is in agreement with others of several polysaccharides (Khounvilay & Sittikijyothin, 2012; Sousa et al., 2015); and it has been associated with structural parameters of polysaccharides such as the distribution of molecular weight, degree of branching, amount and distribution of charged groups (GalA, methyl esters, acetyl groups, and amide).

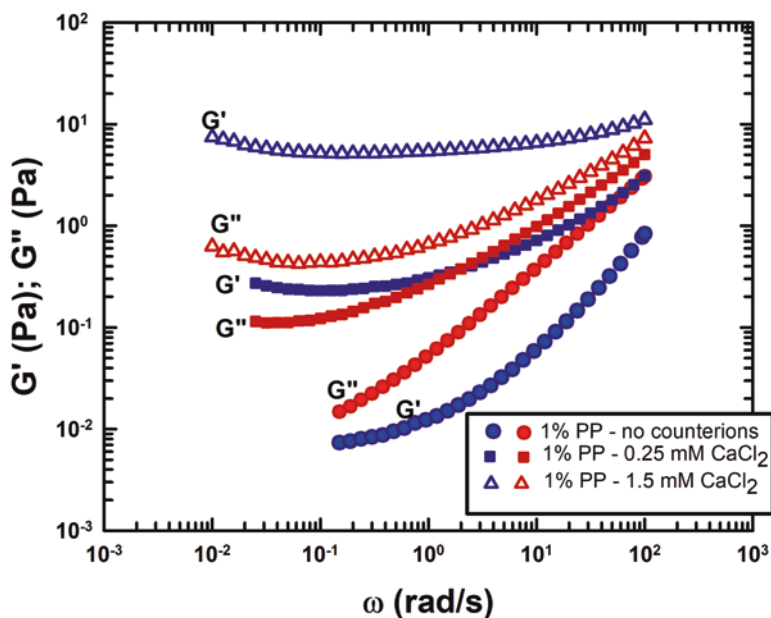
## 5.2 Gelling Properties

The small-amplitude oscillatory shear tests (SAOS) are usually used to examine soft materials' linear viscoelastic behavior. In SAOS experiments, the material is subjected to a sinusoidal shear strain of constant amplitude so that the stress response



is proportional to the input strain, i.e., the strain is so small that the structure of the material does not undergo irreversible damages, and the measured material properties are independent of the input variable. SAOS tests provide the determination of the dynamic moduli: storage modulus ( $G'$ ) and loss modulus ( $G''$ ), as a function of frequency in the linear viscoelastic region (LVR) of the material.  $G'$  and  $G''$  denote the elastic and viscous contributions to the viscoelastic behavior of the tested material, respectively. Therefore, SAOS experiments are valuable tests to examine the gelling behavior of pectins.

As discussed before, pectins from prickly pears of *Opuntia albicarpa* (PP) are low-methoxyl ones; consequently, their gelation was verified through the viscoelastic properties of PP systems as a function of added calcium ions. PP was dispersed into 0.1 M NaCl aqueous solution to exert a shielding effect on the non-esterified GalA units of PP. The sample preparation and details of the rheological tests are described in previous work (Morales-Martínez et al., 2018). Figure 26.6 displays the mechanical spectra of 1% PP in the presence of 0.25 and 1.5 mM  $\text{CaCl}_2$  and without counterions. PP without  $\text{CaCl}_2$  and dispersed into distillate water showed a liquid-like behavior, with  $G''$  higher than  $G'$  and both frequency-dependent. No gel was formed at that pectin concentration without ions. However, the addition of 0.25 mM  $\text{CaCl}_2$  caused a transition in the physical state of 1% PP, a plateau in the  $G'$  and  $G''$  curves appears ( $\omega < 1$  rad/s), where  $G'$  is higher than  $G''$ , indicating a



**Fig. 26.6** Mechanical spectra of 1% pectin from prickly pears of *Opuntia albicarpa* Scheinvar 'Reyna' in the presence of  $\text{CaCl}_2$ . Measurements were carried out at 25 °C within the linear viscoelastic region, using an ARES-G2 rheometer (TA Instruments, USA) and cone and plate fixture (diameter = 40 mm,  $\Delta h = 1$  mm)

transition to solid-like behavior. The mechanical spectrum is characteristic of an incipient gel; the elastic character prevails at low frequencies (or larger observation times) while the viscous one predominates at higher frequencies or shorter observation times. The increase in the calcium ions (i.e., 1.5 mM CaCl<sub>2</sub>) resulted in the formation of more elastic junction zones as can be inferred by the  $G'$  values, at frequencies lower than 1 rad/s;  $G'$  was at least ten times higher than  $G''$ ; after,  $G''$  tends to approach to  $G'$ . That is a typical gel-like rheological behavior. The mechanical spectra reveal the enhancement of the elastic character of PP as calcium ion concentration increased.

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# Chapter 27

## Modern Technologies in *Opuntia* spp. Juice Processing



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**Abstract** Research on the processing of fruits (pears), stems, cladodes, and seeds of *Opuntia* spp. have been recently increased. The fruit of *Opuntia* spp. is considered as health-supporting food due to the variety of bioactive molecules in the fruit. The extraction of fruit juice from the fruits of *Opuntia* spp. is among the studies evaluated in this context. Fruit puree and juice production are one of the essential technologies. Juice extraction from different plant sources uses many techniques based on specific technologies. Some are classified as traditional methods, while others are called “new techniques or novel techniques”, which offer significant advantages to the juice industry. In this field, electrical treatment applications, high hydrostatic pressure, microwave heating, ultrasound, and membrane technique, which serve food research have been introduced in the literature. They have found a chance to be applied in the fruit juice industry but to a differing extent. Some are applied as a pretreatment before juice extraction or used simultaneously with traditional methods or individually, while others are used after juice extraction. These techniques used to provide an increase in fruit juice extraction efficiency and improve the quality characteristics of fruit juice products. These techniques are also utilized to ensure the microbial safety of juices and controlled bioactivity. In this chapter, recent studies on the use of new technologies for processing *Opuntia* spp. juice was evaluated, In addition, the impact of these technologies on the process efficiency and product quality and safety was discussed.

**Keywords** Pear fruit · Yield · Quality · Fruit juice · Pulsed electric field · Ultrasound · Membrane techniques · Microbial safety · Enzyme inactivation

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## 1 Introduction

The *Opuntia* cactus (*Opuntia* spp.) is a large family, a xerophyte of about 200–300 species, and grows mainly in arid and semiarid zones. Due to their remarkable genetic variability, *Opuntia* plants show a high ecological adaptivity and can, therefore, be encountered in places of virtually all climatic conditions (Stintzing & Carle, 2005). Although *Opuntia cacti* originate from Mexico, they are cultivated in both hemispheres and all continents except Antarctica (Inglese et al., 2002). Stem and fruit parts of *Opuntia* spp. are a common topic of recent studies, and they focused on revealing their functionality and nutritional potential for consumers in terms of health-promoting effects and also adding agents for food manufacturing industries.

The berry-like fruit of the variants of the *Opuntia* genus, cactus pear known as prickly pear, is produced by several species of the *Opuntia* genus belonging to the family of *Cactaceae*. Prickly pear is generally ovoid (32–44 cm), has a thin skin, sweet and juicy pulp, and seeds varying in number (from 16 to 518), size (0.25–1.30 cm), and hardness depending on the variant (Reyes Agüero, 2009). Some of *Opuntia* spp. including *O. ficus indica*, *O. stricta*, and *O. dillenii* are commercially grown for food and feed consumption. Different color varieties of *Opuntia* fruits are available (e.g., red, green, violet, and yellow) due to a large genetic variability (Castellanos-Santiago & Yahia, 2008; El Kossori et al., 1998). These fruits are produced mainly in Mexico, with 44% of the World's production or 428,300 t/year, where the San Sebastian Villanueva (Puebla) region is the largest producer of this plant (Jiménez-Aguilar et al., 2015b). Moreover, *Opuntia* spp. fruits are cultivated in other parts of the world, including India, the United States of America, South Africa, etc. (Brutsch & Zimmermann, 1993; Curtis, 1977).

The fruits of *Opuntia* spp. have become popular due to their nutritive value and health benefits, including antioxidant, antiatherogenic, and antiulcerogenic properties, with protective effects against low-density lipoproteins peroxidation (Chang et al., 2008; Ennouri et al., 2007; Galati et al., 2003). Beneficial properties can be attributed to the high antioxidant content of phenolics, betalains, betacyanins, and vitamin C (Chavez-Santoscoy et al., 2009; Fernández-López et al., 2010; Jiménez-Aguilar et al., 2015b; Moussa-Ayoub et al., 2011a; Stintzing & Carle, 2005). Moreover, *Opuntia* spp. fruits contain high concentrations of amino acids, especially proline and taurine, and minerals, including magnesium, calcium, iron, potassium, sodium, and phosphorus (Stintzing et al., 2001). Cactus pear fruit has juicy pulp (28–58% fruit mass) with a large number of seeds (2–10%) enclosed with thick peel (37–67%). Due to these high functional values and health-related properties, *Opuntia* spp. pears are considered valuable fruits in the food industry. For example, they contain colored high-added value compounds (e.g., betacyanins, betaxanthins, etc.) suitable for natural food additives and are generally free from certification requirements (Jimenez-Aguilar et al., 2014; Stintzing et al., 2001). Moreover, these fruits contain mucilage with a high content of galacturonic acids (dietary fiber) that act as thickening agents due to the ability to promote water retention (Matsuhiro et al., 2006; Stintzing et al., 2001).

As mentioned above, since cactus pear fruit is rich in its nutritional and functional potential, it is accepted as a promising future crop for commercial food applications. Although cactus pear fruits have been traditionally utilized for medicinal and cosmetic purposes (Stintzing & Carle, 2005), they are used for the preparation of several food products (e.g., juices, marmalades, etc.) (Barba et al., 2017). There is a couple of the installation of commercial production lines in Mexico, southern California (USA), and Chile, which prove recent studies towards the investigation of cactus pear fruit juice, concentrates, and powders as functional ingredients for the soft drink market (Castellar et al., 2006, 2003; Moßhammer et al., 2005, 2006; Stintzing et al., 2001, 2003).

Cactus pear juice and related products are examined. Prickly pear juice is traditionally processed, and thermal treatment is used to achieve prolonged shelf life for *Opuntia* beverages. However, there is a concern about using heat treatments for food processing and its potential adverse effects on product quality and consumer's health since there is number of studies reporting that heat treatment cause the degradation of thermolabile compounds and change the organoleptic properties of the food product. Thus, the need for the application of innovative non-thermal processing technologies such as high-pressure processing (HPP), high-pressure homogenization (HPH), pulsed electric fields (PEF), and ultrasounds (US) during prickly pear fruit juice production arises (Barba et al., 2017). To this extent, the application of novel technologies in prickly pear fruit juice production must be examined and optimized for possible usage in the food industry.

In this light, a sound knowledge of cactus pears juice's physical and chemical characteristics and potential novel technology usage instead of traditional ones in fruit juice processing should be available. There are some reports about physical, physicochemical properties and some novel processing techniques for prickly pear fruit juice processing. Therefore, the present review summarizes data from the literature and points out promising research areas so far.

## 2 Important Physical and Chemical Characteristics of Cactus Pear Fruits and Juice

In order to reveal the physical and physicochemical properties of any fruit juice, it is required to reveal the juice source's (related fruit/s) properties. The prickly pear fruits offer a broad spectrum of colors from white, yellow, orange, red, and purple based on betalains (Stintzing & Carle, 2005) and contain about 85% water, 15% sugar, 0.3% ash, and less than 1% protein (Mohamed-Yasseen et al., 1996). The thick pericarp is covered with small-barbed spines hosting a juicy pulp with 150–300 non-edible seeds. The latter account for 3–7% on a weight basis, followed by the pericarp and mesocarp (36–48%) and the edible pulp (39–64%) (Felker et al., 2002, 2005; Gurrieri et al., 2000; Hoicke & Stintzing, 1999). Thus, cactus pear fruits may

be divided into three fractions that may be exploited for commercial processing: seeds, peel, and pulp (Moßhammer et al., 2006b).

*Opuntia* spp. fruits are considered health-promoting foods due to the diversity of bioactive molecules found in these fruits. Prickly pears (*Opuntia ficus-indica* L. Mill.) are relevant sources of phytochemicals with proven biological activities and high added-value in the food/nutraceutical industry (Barba et al., 2017; Mena et al., 2018). They are rich in ascorbic acid, betalains (betanin and indicaxanthin), phenolic acids (piscidic acid and hydroxybenzoic acid derivatives), flavonoids (isorhamnetin, kaempferol, and quercetin glycosides), and carotenoids (mainly lutein) (Cano et al., 2017; García-Cayueta et al., 2019; Gómez-Maqueo et al., 2019). Different colors for prickly pear fruits are offered depending on varieties based on betalains covering a broad spectrum from white to purple with pigment contents of 66–1140 mg/kg fruit pulp (Castellar et al., 2003; Stintzing & Carle, 2005). Betaxanthins are responsible for the yellow color, while red is due to betacyanins. The resulted color shades of prickly pear fruits depend on betaxanthin, wherein betacyanin ratios varying between 0 and 11.7 (Butera et al., 2002; Odoux & Dominguez-Lopez, 1996; Stintzing & Carle, 2005).

Mata et al. (2016) identified the composition in organic acids, flavonols, and betalains in the *Opuntia ficus-indica* juice from a region of Portugal. Isorhamnetin derivatives were the dominant flavonol glycosides. A total of nine betalains, including six betaxanthins and three betacyanin were detected in the juice samples wherein indicaxanthin, betanin, and isobetanin were the major pigments. Phenolic acid and phenylpyruvic acid derivatives were also identified (Mata et al., 2016). Betalains and ascorbic acid in prickly pears act as antioxidants by improving the body's redox balance, decreasing lipid oxidation, and acting as radical scavengers (du Toit et al., 2018; Esatbeyoglu et al., 2014). Besides their antioxidant properties, phenolics such as piscidic acid and isorhamnetin glycosides present high anti-inflammatory activity by suppressing free radicals' chain of initiation and propagation, decreasing IL-8 secretion, and reducing NO expression (Antunes-Ricardo et al., 2015; Gómez-Maqueo et al., 2019; Matias et al., 2014).

Due to their betalain and phenolic compound profile, prickly pears have been recently studied regarding their antioxidant and anti-inflammatory activities. However, bioactive compounds in fruits and vegetables possess low bio-accessibility because they must first be released from the food matrix and absorbed in the small intestine (Gómez-Maqueo et al., 2019; Palafox-Carlos et al., 2011).

By processing prickly fruits to fruit juice, pulp, and related products like concentrate, and powder, the fruits are made more affordable value-added products with prolonged shelf life, and high bioaccessibility. Therefore, they deserve more effort to examine possible fruit processing techniques and their effects on the process efficiency and product quality.



### 3 *Opuntia* spp. Juice Processing

Two of the most common domestic uses of cactus pear are juices and pulps. These cactus fruit products require to be examined in terms of the physical, physicochemical properties of fruit juice or pulp and their source fruit, since the utilized techniques for juice processing may have some favorable and adverse effects on the final product. Therefore, in the literature, reports have been published to this extent. In one of them, which was reported by Chavez-Santoscoy et al. (2009), juices from nine prickly pears (*Opuntia* spp.) known as Gavia, Cardon, Amarillo, Pelón, Moradillo, Rastrero, Duraznillo-Blanco, Duraznillo-Rojo, and Tapón were characterized in terms of color, acidity, sugar content, phenolics, flavonoids, betalains, and antioxidant activity and tested *in vitro* against four cancer cell lines. The results indicated that pH's, acidities, and sugar of prickly pears juice varied from 4.27 to 5.46, 0.03–0.27% and 8–14.7°Brix, respectively. The colors of fruit juices were from white to purple, depending on species. The main color pigments (betaxanthins, and betacyanins) were in the range of 3–189 µg/g, and 1.6–300 µg/g, respectively. To figure out the functional potential of fruit juices, their total phenolic, flavonoid contents, and antioxidant capacities were examined, and they varied from 22 to 226 µg gallic acid eq/g, 95–374 µg quercetin eq/g, and 17–25 µmol Trolox eq/mL, respectively (Chavez-Santoscoy et al., 2009). Additionally, *in vitro* cancer, cell viability was determined, and the examined juices showed the highest effects on the viability of prostate and colon cells. The highest flavonoid content was verified for Moradillo, and it diminished both prostate and colon cancer cell viability without affecting other cancer cells. Rastrero reduced the four cancer cell lines' growth without affecting normal fibroblast viability (Chavez-Santoscoy et al., 2009). Although the research points to differences among prickly pears in terms of juice properties and phytochemicals that can prevent oxidative stress and cancer, the results that contribute to scientific research on its potential health-promoting effect are valuable and encouraging further studies in this context.

El Kharrassi et al. (2016) characterized the fruit and fruit juice of 30 accessions of cactus pear (23 accessions of *Opuntia ficus indica* and seven accessions of *Opuntia megacantha*) grown in different regions of Morocco. The pH, titratable acidity, contents of soluble solids, total carotenoids, reducing sugars, and vitamin C in the juices were examined; they were measured as follows: pH, 3.3–4.8; Brix, 6.2°–12.6°; titratable acidity, 0.14–0.88 H<sup>+</sup>/L; total carotenoids, 4.8–20.8 µg/L; and vitamin C, 11.1–29.2 mg/L. The physicochemical characteristics of fruit juices produced from prickly pears of *O. ficus indica* and *O. megacantha* from Morocco did not significantly differ. However, the results pointed out the importance of country-based differences in cactus pears of *Opuntia* spp. (El Kharrassi et al., 2016).

To evaluate fruit-originated products, besides their nutritional values, health-promoting effects should also be focused on, like antioxidant potential. Dehbi et al. (2014) studied the physical and chemical compositions such as moisture, sugar content, total fibers, protein, ash, pH, acidity, minerals contents, and total phenolic contents of nine prickly pear juices. It was revealed that besides being rich in

micronutrients, their phenolics are antioxidants that contribute to the nutritional prickly pears quality (Dehbi et al., 2014). Madrigal-Santillán et al. (2013) led a study about the antioxidant capacity of three varieties of prickly pear juice (red-purple, white-green, and yellow-orange) in five different concentrations (100, 250, 500, 750, and 1000 mg/mL) by DPPH· (1,1-diphenyl-2-picrylhydrazyl radical) method, selecting the best variety to determine its anticlastogenic potential against methyl methane sulfonate (MMS). Fruit juice produced from the prickly pear red-purple variety (PPRP) was had the highest antioxidant capacity in its all studied concentrations. Therefore, its anticlastogenic potential was examined with a micro-nucleus assay. PPRP juice does not show any genotoxicity; on the contrary, it may reduce the number of micronucleated polychromatic erythrocytes (MNPE). In this regard, the PPRP showed an anticlastogenic effect directly proportional to its concentrations. Thus, the highest protection was obtained with a concentration of 25 mL/kg after 48 h treatment (Madrigal-Santillán et al., 2013).

There are older studies in the literature. Paredes and Rojo (1973) studied prickly pear juice in one of the pioneering studies. In their studies, the pH of cactus pear juice was adjusted to 4 using citric acid, and sodium benzoate was used as a preservative. Subsequently, the fruit juice's microbial stability was achieved with canning under vacuum following a mild thermal treatment at 90 °C for 5 min. The organoleptic properties of the product were found to be satisfactory. However, in another study (Espinosa et al., 1973), some difficulties were encountered in terms of stability in *Opuntia ficus indica* juice production.

In the fruit juice industry, the concentrate is one option for manufacturers since they have higher stability, better microbial safety, and can be stored under easier storage conditions with more compact storage facilities than juices. Therefore, fruit juice concentrates should also be taken into account in scientific research. As an example, Almendares (1992) analyzed the concentrate of cactus pear juice. According to his report, the reduction in the concentrates' water activity compared to fresh prickly pear juice provided microbial safety, which meant a long shelf-life. In that study, the Brix value of the concentrates produced in a vacuum evaporator operating at 40 °C was measured in the range of 63–67°Brix. However, in addition to being advantageous, it is well known that the evaporation process leads to loss of aroma and degradation of color pigments (Sàenz, 1996; Sáenz et al., 1993). For these possible reasons, the sensory score of concentrated prickly fruit juice samples in the study was found to be low (being 5 in 1–9 points scale) (Saenz, 2000).

Thermal treatments are common applications in the food industry to prolong the shelf-life by decreasing microbial load and inactivating spoilage enzymes (pasteurization, sterilization, etc.). However, thermal treatments are not such innocent since exposure to high temperatures may damage nutritive value and natural texture, taste, flavor, and color in foods. The adverse effect of thermal treatments on foodstuffs' quality is a challenging but not avoidable problem for food manufacturers. Thus, in order to find alternative methods to replace heat treatments, there are increasing efforts and the industry and academy provide funds and expertise human source for those efforts. One of the adverse effects of thermal treatment is the color deterioration, and resulting color changes, quality loss, the occurrence of health-threatening

molecules. To avoid the degradation of color pigments in food products, different methods have been developed. Some are based on non-thermal treatments, whereas some use mild-heat treatments, including precautions and/or additives. It is well known that the main color pigments in prickly fruit juice are betalains, and their retentions in fruit juice after thermal treatments are a good indicator for process success and product quality. To this extent, overall color and betalain retention in yellow-orange cactus pear (*Opuntia ficus-indica* [L.] Mill. cv. 'Giulla') juice was examined after thermal treatment at varying temperature for 5–60 min operation (Moßhammer et al., 2006a, b, c). A 24-h color regeneration period was investigated. The effect of temperature was investigated at 75, 85, and 95 °C. The isoascorbic acid of 0.1% was utilized before heating to control color alteration and betalain degradation. Moßhammer et al. (2006a) suggested the addition of 0.1% isoascorbic acid, which significantly delays both betaxanthin and betacyanin degradation upon heating resulting in improved color stability.

The ratio of indicaxanthin/isoindicaxanthin was identified as a parameter to calculate the initial betaxanthin content and, thus, the extent of overall pigment degradation. The formation of 2-decarboxy-betainin was found as an indicator for harsh heat exposure, and a 0.1% isoascorbic acid dosage used before treatment also limits its occurrence. Moßhammer et al. (2006a) also conducted storage experiments for over 6 months. The presence of light was crucial, and storage in the dark exhibited notably better color and pigment retentions in samples than those illuminated (Moßhammer et al., 2006a). Besides, Moßhammer et al. (2007) investigated the impact of added ascorbic, isoascorbic, and citric acid on cactus pear betalains' heat stability at pH 4 and 6, respectively. To differentiate the cactus pear juice matrix's effects on pigment stability, juice, and purified pigment preparations were evaluated separately. Pigment stability was found to vary depending on the type and concentration of the respective additive and pH conditions. Although, the addition of ascorbic, isoascorbic can significantly stabilize color pigments (betaxanthins and betacyanins) in yellow-orange cactus pear juice as well as in purified pigment preparation and citric acids, due to supporting effects of matrix compounds such as sugars, organic acids, amino acids, and pectic substances, cactus pear juice provided a better medium for the stabilizing effects of the additives compared to purified preparations due to the supporting effects of matrix compounds. The maximum pigment retention of 79% was attained by adding 0.1% citric acid into fruit juice at pH 6 (Moßhammer et al., 2007).

All the reasons mentioned above directed the food science research towards developing novel (non-thermal) technologies able to replace conventional thermal processing. The impact of innovative processing technologies such as high technology membrane techniques, high hydrostatic pressure (HHP), pulsed electric field (PEF), and ultrasound (US), on the bioactive compounds' recovery (e.g., phenolics and anthocyanins) from different red fruit products (e.g., strawberries, chokeberry, blueberries, pomegranate, etc.), has been evaluated by several authors (Bursać Kovačević et al., 2015, 2016a, b; Silva et al., 2016; Zinoviadou et al., 2015). Barba et al. (2017) summarized the effect of process techniques on *Opuntia* fruits and their derived products since they strongly influence their phytochemicals' stability.

Authors point that novel non-thermal technologies are efficient in recovering high added-value compounds from *Opuntia* fruit, its products, and by-products/waste. Overall, high-pressure processing and pulsed electric field technology have emerged as promising methods to extend *Opuntia* beverages shelf life, and supercritical CO<sub>2</sub> extraction as a useful tool to extract oils (Barba et al., 2017).

Enzyme usage is a common application for the fruit juice industry regarding its direct effects on juice yield and product quality. Essa and Salama (2002) evaluated pectinase and cellulase enzymes' potential usage to examine efficacy for improving juice yield, stability, and quality from prickly pear fruit. It was reported that juice yield from prickly pears increased with pectinase addition into the process, and the resulted fruit juice has stable color, color-assayed as the release of anthocyanins or carotenoids, and clarity of the juice. The authors pointed out the importance of pectinase's concentration level varying from 0.05 to 0.50% v/w. Although higher juice yield was obtained from fresh fruit with a 0.25% v/w of pectinase addition, accompanying bitter flavor came out. Among three pectinase and cellulase concentrations, pectinase at 0.50% v/w produced a higher yield, a sediment-free clear juice, and high-quality juice. With improvements in the membrane technologies, its usage creates a new perspective for fruit processing.

Serving the usage of the membrane system, Castro-Muñoz et al. (2018) carried a study in which the effect of ultrafiltration (UF) operation on the physicochemical composition of *Xoconostle* juice from native Mexican fruits was investigated. Additionally, the physicochemical properties of prickly pear juice for the characterization of the nutritional and functional properties were investigated to show the clarification process's effect. The proposed method resulted in a satisfied clear juice (3.79 NTUs) with high functional potential and satisfactory enhancement in color properties. The average total phenolic and betalain fruit juice contents were found as 27.5 mg gallic acid/L, and 18.8 mg/L, respectively. Juice samples were also reported to have high antioxidant activity (21.4 TEAC) (Castro-Muñoz et al., 2018). Similarly, for clarification of cactus pear juice, a microfiltration membrane was used in a study carried by Vergara et al. (2015). In this study, two different types of the membrane (ceramic and polymeric) at pore size of 0.2 μm were utilized to assess their effects in terms of the clarification performance and the retention of color pigments, betalains. Retentates from the filtration system were clear and had low turbidity and high betalains retention (20%). The best separation was obtained using the ceramic membrane (Vergara et al., 2015).

Foodstuffs may be susceptible to spoilage due to their structure suitable for biochemical reactions and/or microbiological activities. Therefore, the food industry devotes large amounts of funding and spends efforts to deal with these issues. One of the ways to solve these problems is the thermal processing, especially if the foodstuff contains sufficient nutrients and has suitable environmental conditions such as high moisture content and low acidity, severe heat treatment is required. Cactus pear fruits are counted in this group due to their high soluble solid content and low acidity value. Therefore, the pulp or juice must be subjected to a severe heat treatment (115.5 °C or higher) to limit and/or stop those undesirable changes/microbial invasion (Cassano et al., 2007). However, besides microbial safety, a relatively long

thermal treatment causes an unattractive hay taste, and the final product does not resemble the original fresh juice due to changes in color and flavor. Cassano et al. (2007) conducted a study about the potential use of a membrane-based process to clarify and the concentration of the cactus pear fruit juice, instead of enzyme and thermal treatments. Ultrafiltration (UF) and osmotic distillation (OD) were two successive methods to clarify and concentrate the cactus pear juices. First of all, fresh fruit juice with a total soluble solids (TSS) content of about 11°Brix was clarified during the UF step. The clarified cactus pear juice was concentrated up to a TSS content of 61°Brix at 28 °C by OD.

The clarified and concentrated juice samples were examined to determine its total antioxidant activity (TAA). The ascorbic, citric, and glutamic acid, betalains contents, and viscosity evaluate the effects of the membrane processes on the juice's quality and composition. The clarification and concentration of the cactus pear juice using UF and OD techniques represent a valid approach to process the juice at low temperatures preserving the organoleptic, nutritional, and sensorial characteristics of the fresh fruit. Analytical results of the UF process confirm the possibility of recovering the most ascorbic, glutamic, and citric acid in the clarified juice. In conclusion, the UF/OD integrated process was evaluated as a combined method to preserve the nutraceutical and functional importance of the cactus pear fruit juice concerning the traditional clarification and concentration procedures (Cassano et al., 2007). Another project based on membrane technologies focused on microfiltration (MF) and ultrafiltration (UF) processes to reveal their effects on the physicochemical composition of the cactus pear juice produced from fruits of Italian (Sicily) origin (Cassano et al., 2010). The results indicated that the clarified cactus pear juice was comparable with its fresh one in terms of physicochemical and nutritional properties, but the absence of suspended solids and betalains remained concentrated in the retentate. Authors also suggested that the retentate, being a fraction enriched in fibers, sugars, and betalains, can also be used to prepare different food formulations (Cassano et al., 2010).

As mentioned above, alternative products like concentrates, powders from cactus pear juice provide an extending shelf life and cause added value. Moßhammer et al. (2006b) aimed to develop an alternative process for producing juice concentrates and powders from cactus pear. Since color pigments are susceptible to heat treatments, as an alternative to HTST pasteurization, cross-flow microfiltration was applied for non-thermal cactus pear juice preservation. Juice concentration and power productions were achieved by rotary evaporation and freeze-drying at laboratory scale and compared to products obtained at pilot plant-scale applying a three-stage column evaporator and spray drying, respectively. Color and some quality parameters were monitored to be able to evaluate the process performance of proposed techniques. Initial color features could be protected for both juice concentrates and fruit powders. No adverse effect on the quality of juice concentrate or powder was reported.

One alternative technique as a replacer of thermal treatments or making thermal treatments as mild is ultrasound application. Ultrasound is briefly sound waves created at a specific frequency and used for different purposes like medical

applications, food manufacturing treatments, etc. One study aimed to examine the effect of ultrasound on purple cactus pear juice (Zafra-Rojas et al., 2013). Ultrasonication was carried at different ultrasound conditions at amplitude levels (40% and 60% for 10, 15, 25 min; 80% for 3, 5, 8, 10, 15, and 25 min). Treated cactus pear juice samples were assessed in terms of the quality (stability, °Brix, pH), microbial growth, total phenolic compounds, ascorbic acid, and antioxidant activity (ABTS, DPPH·, and % chelating activity). A time period of 15–25 min application of ultrasound reduced the microbial load of juice samples, but it did not cause any significant change in the samples' quality and antioxidant capacity. The results were concluded that the proposed ultrasonication is a promising technique for the safety of purple cactus pear juice with preventing quality characteristics (Zafra-Rojas et al., 2013).

As there are many other species of *Opuntia* spp., there might be some promising alternatives to *O. ficus-indica*. Some of these species are *Opuntia dillenii* and *Opuntia macrorhiza*, providing edible fruits. In this context, Moussa-Ayoub et al. (2016) studied the effect of pulsed electric fields (PEF) as a non-thermal process on important juice characteristics. Promising results have been reported compared to the use of microwave heating and pectinases. *Opuntia dillenii* cactus fruits were evaluated in terms of fruit juice production, and cactus juice exhibited the desired technological characteristics. The yield was increased with a moderate electric field treatment of the fruit mash along with the improved juice properties. Additionally, juice functionality was evaluated and reported to contain high amounts of phenolics accompanying antioxidant activity (Moussa-Ayoub et al., 2016). Similarly, Moussa-Ayoub et al. (2011b) included the fruits of *Opuntia macrorhiza* in their study to assess its juice potential, as it is a good source of flavonols, betacyanins, and vitamin C. This study aimed to improve the yield and juice characteristics by taking the advantages of some pre-extraction techniques. Experimental trials included four different methods and/or their combinations (PEF, heat treatment using microwaves, enzymatic treatment with commercial pectolytic enzymes, and a combination of thermal and enzymatic treatments). A significant improvement in juice yield was achieved from the mashes treated enzymatically or with a combination of thermal and enzymatic treatments. The production of juice from the PEF-treated mash provides promising effects in terms of functional properties by increasing the release of flavonols into the juice. However, it remains lower than that achieved enzymatically or by a combination of thermal and enzymatic processes. PEF is considered a faster and more energy-efficient method to enrich fruit juice with bioactive substances.

On the other hand, betacyanins, an important group of color pigments, can be preserved by the PEF treatment. At the same time, significant reductions have been observed in fruit juice samples produced by the thermal-based pre-extraction process or in combination with enzymatic degradation/modification of those pigments' structures (Moussa-Ayoub et al., 2011b). Similar to its functional bioactive content, *O. macrorhiza* fruit has been reported to exhibit lower antioxidant activity in heat treatments, partially due to functional bioactive degradation. There was no significant difference in the juice samples' antioxidant activity obtained by other pre-extraction methods, including PEF treatment. In the same study, following fruit

juice extraction, 3 min of pasteurization at 95 °C, PEF at 35 kV/cm until loading energy of 85 kJ/kg, or 10 min of High Hydrostatic Pressure (HHP) at 600 MPa were found to be required to ensure microbial safety for juice samples. Pasteurized juice's antioxidant activity was slightly lower than juices preserved by PEF and HHP techniques (Moussa-Ayoub et al., 2011b). High-Pulsed Electric Fields (HPEF) treatment was investigated to determine the effects on extraction yield and juice quality of the endocarp of nine varieties of prickly pears (*Opuntia* spp.): 'Tapón Aguanoso', 'Orejón', 'Cacalote', and 'Burra la Cruz', known as purple varieties, and 'Rojo Pelón', 'Cardona', 'Sangre de Toro', 'Sandía', and 'Rojo Insurgentes', known as red varieties (Jiménez-Alvarado et al., 2015). The extraction yield and their juices' overall quality in terms of betalains and phenolics and their antioxidant activity and color were evaluated. The results showed that the HPEF treatment favored extraction yield and the examined juice characteristics, but to a varying extent depending on species. In terms of extraction efficiency, HPEF was found to have the highest effect on prickly pear fruits of the 'Sandía' variety. Juice extracted from this variety was increased from 4.48% without HPEF treatment to 52.2% (approximately 11.5 times) with HPEF application. The favorable influence of HPEF has also been seen for the quality characteristics and color stability of prickly fruit juices. The content of betalains, phenolics, and antioxidant activity increased in fruit juices when HPEF was applied. Color stability was evaluated against changes in pH and temperature, and the highest stability was observed for juice samples of 'Orejón' and 'Tapón Aguanoso' varieties. Therefore, it has been concluded that HPEF can serve to extract juice from *Opuntia* spp. fruits with high yields and recovering valuable compounds and improving the overall quality (Jiménez-Alvarado et al., 2015).

Ultrasound is another novel technology that is common in food processing studies because it produces promising results. This study is an example of using ultrasound in the production of juice from green cactus pear fruits (Cansino et al., 2013). Ultrasound therapy at three amplitude levels (40%, 60%, and 80%) and three-time periods (10, 15, and 25 min) were evaluated to determine the process effects on the quality (pH, °Brix, stability), microbial growth, phenolic content, ascorbic acid, and antioxidant activity of green cactus pear juice. It has been reported that the initial microbial load of the juice can be controlled at high amplitude levels with longer process time, mostly higher than 60% amplitude level for 15 min of treatment time. Total soluble solid content may exceed the level of 13°Brix in case of US treatment for longer than 15 min. The juice's physical stability was achieved by treatment at 60% for 15 min, at 80% for 8, 15, or 25 min. The functional potential was also evaluated by Cansino et al. (2013). In the US, a satisfactory amount of phenolic was released into the fruit juice when 80% was applied for 25 min, while treatments of 60% for 25 min and 80% for 10 min resulted in higher vitamin C content by Cansino et al. (2013). These results were accompanied by the antioxidant potential of the juice samples (Cansino et al., 2013). Another technology used to produce minimally processed healthy foods is HHP used for non-thermal microbial inactivation. Jiménez-Aguilar et al. (2015a) conducted an HHP study to investigate the effect of this technology on prickly pear beverages in terms of vitamin C content, phenolic compounds, flavonoids, and betalains as well as antioxidant activity. In this study,

as a hurdle technology, beverages also formulated with acids and preservatives were studied under the same HHP conditions. For two *Opuntia* varieties, beverages were prepared as 10% peel and 90% pulp either with (A means beverage with additives) or without (N means beverage without additives) the incorporation of acids and antimicrobials (0.3 g sodium benzoate, 0.15 g sodium sorbate, 1.4 g fumaric acid, 0.4 g tartaric acid and 0.3 g sodium citrate per liter of a beverage). Samples were treated at 400 and 550 MPa for different periods. As a thermal process, heat sterilization at 131 °C for 2 s was performed, and samples did not include any additives. The superior effect of HHP on total phenolic and antioxidant activity was observed for prickly pear juices when treatment was performed at 550 MPa for 2 min. Color pigments (betaxanthins and betacyanin) content was also increased with HHP at 550 MPa for 2 min. Heat sterilization resulted in significant decreases in vitamin C, total phenolic, flavonoid, color pigment contents of juice samples as well as a reduction in antioxidant activity (Jiménez-Aguilar et al., 2015a).

## 4 Microbial Safety

Consumers are increasingly demanding minimally processed healthy foods with more natural flavor and color, high quality, and long shelf life. Therefore, improving the quality of food products and developing the appropriate technologies for food preservation is crucial. Although an efficient inactivation of spoilage or pathogenic microorganisms can be achieved, thermal treatments might have a negative impact. In the meantime, also emerging and innovative non-thermal preservation technologies such as PEF, HHP, cold atmospheric plasma, or ultrasound are increasingly considered for preserving food (Barba et al., 2012, 2015; Knorr et al., 2011).

It is well-known that for foodstuffs, microbial safety is of paramount importance and is one of the main challenges facing manufacturers to this extent. Therefore, significant efforts have been made to overcome this problem. Until now, thermal processes have been used as an important tool to deal with them. However, heating foodstuffs provide microbial safety and cause problems like degradations, reductions in nutrients, and loss of quality parameters. For this reason, there are many studies aimed at ensuring the microbial safety of foodstuffs with different technologies. The use of some novel technologies to ensure microbial safety of prickly pear juices were reported. Cruz-Cansino et al. (2015) compared the samples of *Opuntia ficus indica* juice untreated, pasteurized, and subjected to thermoultrasonicated. The latter was performed using a 1500-W ultrasound device at a fixed frequency of 20 kHz for 15 and 25 min at 80% amplitude. Pasteurization was done at 70 °C in 30 min. Thermoultrasonication improved color stability and increased juice viscosity, while significantly reducing the solid sediment content of fruit juice. Total plate counts decreased from the first day of storage, exhibiting values of 1.38 and 1.43 log CFU/mL, for 15- and 25-min treatment. Compared to the control, both treatments reduced enterobacteria counts (1.54 log CFU/mL), and compared to pasteurized juice decreased pectin methylesterase activity (3.76 and 3.82 UPE/mL),



maintained high values of ascorbic acid (252.0 and 257.1 mg AA/L) and antioxidant activity (by ABTS: 124.8 and 115.6 mg VCEAC/100 mL; and DPPH: 3114.2 and 2757.1  $\mu\text{mol TE/L}$ ). During storage, thermoultrasonicated juices had a minimal increase in pectin methylesterase activity (from Day 14) and exhibited total plate counts similar to pasteurized juice. Thus, it can be concluded that thermoultrasonication is a promising technique for preserving cactus pear juice by inactivating the PME enzyme and maintaining its antioxidant properties. However, to make a clear statement about juice preservation, more efforts are required to evaluate the microbial inactivation effect (Cruz-Cansino et al., 2015).

Another ultrasound study was conducted by Cansino et al. (2013) to examine its effects on the microbial load of green cactus pear juice. The control sample loads of TPC and Enterobacteria were 4.6 log CFU/mL and 4.2 log CFU/mL, respectively. Total and enterobacteria counts were reduced below the detection level in the long term. Amplitude and time were determined the most significant parameters of ultrasound application for microbial inactivation for green cactus pear juice samples. Amplitude levels of 60% and 80% were found to be enough for complete inactivation in the case of long-term treatments >15 min. Cansino et al. (2013) explained the mechanism under the effect of ultrasound in microbial inactivation mainly as temperature effect, since its level in the juice samples reached 58–66 °C for 15 min and 25 min, respectively, when amplitude was 60%. Those temperature levels risen to 71 °C and 76 °C for 15 min and 25 min treatments at an amplitude of 80%. Therefore, there is greater effectiveness of inactivation. Another mechanism playing a role in the effectiveness of ultrasound for microbial inactivation is cell destruction occurring for long-term applications due to cavitation (Bhat et al., 2011). Besides, studies suggest that microorganisms subjected at temperatures >50 °C show greater sensitivity to the effect of cavitation that causes the weakening of the bacteria membrane (Patist & Bates, 2008; Sala et al., 1995; Villamiel & de Jong, 2000). Cruz-Cansino et al. (2016) led a study to examine the inactivation of *Escherichia coli* inoculated into cactus pear juices (green and purple) and evaluated the effectiveness of ultrasound application for the storage period. Total inactivation was observed in fruit juice samples after 5 min of ultrasound treatment at high amplitude levels 70% and 90%; however, it was not seen for 60% and 80% amplitude levels. Bacterial growth was seen in all cactus pear juices after 1 and 2 days of storage. At 90% amplitude for 5 min, ultrasound treatment resulted in non-detectable levels of *E. coli* in cactus pear juice up to 2 days of storage. The authors also reported that there was not any significant change in physical properties (pH, titratable acidity, and soluble solids) of investigated fruit juice samples during these applications (Cruz-Cansino et al., 2016).

Koubaa et al. (2016) evaluated the potential of the non-conventional pretreatments, pulsed electric fields (PEFs), and ultrasounds (USNs) to enhance the extraction of red colorants from red prickly pear (*Opuntia stricta* Haw.) peels and pulps. Results showed that PEF + SAE, and USN + SAE enhanced the extraction of red colorants (betanin/isobetanin) compared to untreated tissues.

## 5 Conclusion

*Opuntia* spp. have high potential as a raw material for fruit juice production, although having some challenges. There is a demand to find a way as a solution to these issues. Novel technologies may give support to the food industry with its advantages. Reports exhibited the significant impacts of some novel technologies on prickly pear fruit juice production lines at different steps, including juice extraction, enhancement in quality characteristics, and ensuring microbial safety and extended shelf life. However, there is a need to conduct more studies serving on this topic. The current work may help researchers light up their ways towards these targets and find missing points required to be scientifically considered in their studies. This work is designed to collect published reports to explain the current situation about using novel technologies in prickly fruit juice production in terms of different production steps and the storage period. Meanwhile, it is a picture that also shows areas where more effort is needed to adapt new techniques in prickly pear juice production.

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# Chapter 28

## Novel Technologies in Juice Processing from *Opuntia* spp. Fruits



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**Abstract** Juice processing and preservation have been regarded as age-old technologies. *Opuntia* spp. is a fruit that grows in a semi-arid region and marginal soils and does not require much attention for cultivation. In view of the fruits' nutritional and sensory attributes, unique juice processing technologies have been developed for higher yield and a prolonged shelf-life. The current chapter discusses the traditional juice processing methods for *Opuntia* juice extraction. Also, it depicts the use of novel technologies such as ultrasound, pulsed electric fields, and high hydrostatic pressure for *Opuntia* juice processing. The advantages of the modern processing and the current status of the juice processing from the fruits of *Opuntia* spp. have been elaborated in this chapter.

**Keywords** Beverages · Preservation · Health perspective · Ultrasound · Pulsed electric fields · High hydrostatic pressure · Packaging · Microbiological quality

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## 1 Introduction

*Opuntia* spp. is the largest genus of the family Cactaceae, which comprises various species and cultivars (del Socorro Cruz-Cansino et al., 2015). These are also known as cactus pear plants or nopal (Moussa-Ayoub et al., 2011a, b). Because of the resourceful water utilization potential, the crop is spread in abundance in the arid and semi-arid geographical zones (Sáenz et al., 2004; Aruwa et al., 2018). On the other hand, the crop is extensively distributed in Mexico, its origin, and other corners of the globe, including Southern America, the Mediterranean basin, and the Middle East. The plant bears edible green stems, known as cladodes and fruits (Moussa-Ayoub et al., 2011a). The fruit has gained interest because of its nutritional and healthful biochemical constituents (Verón et al., 2017). Fruit of *Opuntia* spp. is regarded as a functional food because of its composition and is being processed for different products (Joubert, 1993; Aruwa et al., 2018).

The juice extracted from *Opuntia* fruits is an essential sugar source such as glucose and fructose, minerals, prebiotic fiber, and several antioxidative biochemicals (e.g., vitamins C, tocopherols, carotenoids, and flavonoids) (Verón et al., 2017). The juice processing technology includes treatments like enzymatic and thermal processes, which increase the juice yield. In contrast, nonthermal processing saves energy and processing time and retains more bioactive components from the fruit's pulp into the juice. The frequently preferred nonthermal techniques are ultrasound, pulsed electric fields (PEF), and high hydrostatic pressure (HHP), as these techniques do not negatively impact the physicochemical structure and sensitive bioactive compounds such as vitamin C and betacyanin (Sahu & Panda, 2018). The study of Moussa-Ayoub et al. (2011b) reported *Opuntia* juice with a higher antioxidant property after processing with PEF and HPP juice compared to traditionally processed (thermally treated) juice. The most innovative and emerging nonthermal food technologies such as PEF, HHP, cold atmospheric pressure plasma are modern processes for improving *Opuntia* juice yield, its flavonol content, and improved juice characteristics.

## 2 Process of Juice Making form *Opuntia* spp.

Several innovative technologies are exploited to enhance the shelf-life and carefully safeguard the dietary and organoleptic attributes of *Opuntia* juices (del Socorro Cruz-Cansino et al., 2015). The general preparation process has been presented in Fig. 28.1.

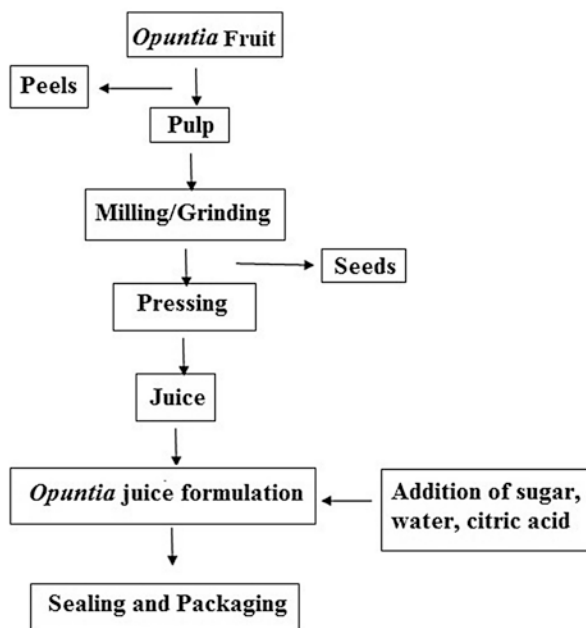


Fig. 28.1 Preparation and processing of *Opuntia* juice

## 2.1 Plant Material and Preparation of Extract

The fruits are usually preserved in low temperatures (8–12 °C) after the harvesting and before juice processing. The fruits are cleaned in running water and deskinning by peeling the rinds before lyophilization (del Socorro Cruz-Cansino et al., 2015).

## 2.2 Pulp Making and Seed Separation

The fruit comprises a red or purple rind with a thin layer of skin and a pulp full of small, hard seeds (Moussa-Ayoub et al., 2016). *Opuntia* peel comprises 60% of the whole fruit and contains fatty acids, vitamins, carbohydrates, and antioxidants (Aruwa et al., 2018). They contain a considerable amount of fiber (~32.6), pectin (~14%), and ascorbic acid (~87%) (Smeriglio et al., 2019). The rind and skin are peeled out and discarded during the juice processing and considered a by-product for further processing (Aruwa et al., 2018).

The fruit pulp of *Opuntia* spp. is enriched with bioactive constituents such as vitamins, carotenoids, and phenolics (Koubaa et al., 2016). Pectin, mainly responsible for the pulp's viscosity, is a positive element towards the production of juices. The betalains impart color as well as functional activity to the pulp. Betalains are

more stable than chlorophylls under thermal treatment and pH variation (Saenz, 2000). The pulp has a higher pH range, i.e., 5.6–6.5, compared to other fruits, and TSS ranging from 11°Brix to 17°Brix, which makes it susceptible to microbial degradation (Moussa-Ayoub et al., 2011a). The acid/sugar ratio should be in an appropriate range for the organoleptic acceptance; hence it is a challenge for the fruit processing (Moussa-Ayoub et al., 2011a).

### 2.3 Preparation of *Opuntia* Juice

The fruits are washed and air-dried at 40 °C overnight and are peeled manually. Samples are chopped into small pieces and further homogenized by a blender cum strainer (stainless steel) to separate the seeds and mesocarp fibers. The juice is preserved at –20 °C until use (Verón et al., 2017), and the procedure is repeated as many times as necessary to homogenize the whole fruit batch (Castañeda-Yañez et al., 2018). The juice is pasteurized by heating (64 °C for 30 min) commercial batches of *Opuntia* juices (Verón et al., 2017). The simple acidic taste with desirable color makes the juice highly preferable for blending with other highly-flavored juices, such as strawberry juices (Moussa-Ayoub et al., 2016).

### 2.4 Preservation of the *Opuntia* Juice

Some researchers have reported using several parameters/processes for the preservation and prevention of microbial spoilage of the *Opuntia* juice (Paredes & Rojo, 1973; Cassano et al., 2010). The use of citric acid to lower the pH to 4.3, sodium benzoate (500 ppm), and thermal treatment for 5 min at 90 °C are the examples that showed the preservation of pleasant flavor taste and without microbial interference juice. Espinosa et al. (1973) studied the use of lemon juice with thermal treatment (80 °C for 20 min), acetic acid fermentation, and vacuum canned in the enameled tin to preserve *Opuntia* juice. Another option related to *Opuntia* juices was to produce fruit concentrate (Trivedi & Raval, 2017). The lower  $a_w$  value of the concentrates compared to the juices provides microbial inhibition and extends the shelf-life of the product.

## 3 Fruit Juice from *Opuntia* spp. in Health Perspective

Juice of the fruit of *Opuntia* spp. is rich in essential nutrients and health-promoting phytochemicals, making it a healthful drink. The juice contains sugars, mostly glucose and fructose (12–17%); it also contains vitamins (vitamin A, C, and E), phenolic compounds, and selected amino acids (Yahia & Saenz, 2011). The pigments

found in different cultivars of cactus fruits, namely betaxanthins (yellow) and red betacyanins (red), impart antioxidant properties to the juice. The essential minerals present in the fresh fruit juice include calcium, magnesium, potassium, and phosphorus (Jana, 2012). An interesting study was conducted by Madrigal-Santillan et al. (2013) to understand the antioxidant potential of different varieties of prickly pear juices. It was observed that the red-purple variety showed higher DPPH· antioxidant property as compared to the yellow-orange and the white-green variety. Further, the juice obtained from the red-purple variety showed a strong anticlastogenic effect against the mutagen methyl methanesulfonate in the mice model. It was observed that the effect is directly proportional to the concentration of the juice. Out of the different doses of the juice, i.e., 25, 16.5, and 8.3 mL/kg in the mutagen presence, the highest protection was observed at 25 mL/kg.

Prickly pear juice was also studied to understand the effects of diabetic cataract rats. The animal models were divided into groups to understand prickly pear juice's impact on diabetic cataract rats (Abd El-Razek et al., 2012). Alloxan was injected to induce diabetes, and further, they were orally fed with 1–4 mL concentrated juice per rat per day. It was concluded that a considerable fall in the glucose in blood and lens was observed in the mice treated with cactus juice for 8 weeks. Cactus pear juice has also shown some promising results in human clinical studies. Twenty-two volunteers were considered for the study, out of which 11 were experimental groups, and the rest 11 were control groups (Khouloud et al., 2018). Yo yo intermittent recovery test was conducted before and after 2 weeks of prickly pears juice consumption. It was concluded that the volunteers with the juice consumption showed a noteworthy low content of total cholesterol, triglyceride, low-density lipoprotein, and monoaldehyde as compared to the control group (volunteers without supplementation). Further, it was observed that the supplementation of the juice could lead to better performance in the yo-yo intermittent recovery test.

## 4 Novel Technologies in *Opuntia* spp. Juice Processing

Novel technologies (thermal and nonthermal) have been introduced by the food industry or researchers to reduce energy cost, preserve, and improve food quality, especially in fruit processing (Nielsen et al., 2009). Thermal processing is generally the most widely accepted preservative method for extending the shelf-life of fruits and their juices but sometimes could impair its nutritional qualities (Tiwari et al., 2009a). As indicated in Table 28.1, available literature indicates that limited studies are subjecting *Opuntia* fruit and its products to these novel technologies such as hydrothermal treatments, HHP, ultrasound, and PEF.

Usually, thermal treatment is conducted to reduce the microorganisms and deactivate the spoilage enzymes in *Opuntia* beverages (Barba et al., 2017). However, the thermal treatment of *Opuntia* beverages has been reported to cause alteration in the organoleptic and functional properties of the product and the degradation of thermolabile compounds (Barba et al., 2017). A study on the thermal treatment in

**Table 28.1** Novel technologies used in producing juice from *Opuntia* spp.

Specie used	Novel technology/ treatment	Conditions	Reference
Thermal			
<i>O. macrorhiza</i>	Microwave heating	90 °C and 324 kJ/kg	Moussa-Ayoub et al. (2011b)
Nonthermal			
<i>O. ficus-indica</i>	Thermoultrasound (ultrasound and mild temperatures)	20 kHz at 80% amplitude for time periods of 15 or 25 min using a probe of 13 mm	Cruz-Cansino et al. (2015)
<i>O. ficus-indica</i>	Ultrasound	20 kHz constant frequency with 60%, 70%, 80%, and 90% amplitude levels for 1, 3, and 5 min	Cruz-Cansino et al. (2016)
<i>O. albicarpa</i> and <i>Opuntia ficus-indica</i>	PEF	36 kV cm <sup>-1</sup> electric field strength with 25 and 50 Hz and pulse widths of 11, 13 and 15 µsec	García-García et al. (2015)
<i>O. ficus-indica</i>	HHP	400 MPa for 1, 2, 4, 8, and 16 min, and at 550 MPa for 0.3 (20 sec), 0.7 (40 sec), 1, 1.5, 2, 2.5, and 4 min	Jiménez-Aguilar et al. (2015)
<i>O. macrorhiza</i>	PEF	3 kV/cm, 85 kJ/kg	Moussa-Ayoub et al. (2011b)
<i>O. macrorhiza</i>	HHP	600 MPa for 10 min	Moussa-Ayoub et al. (2011b)
<i>O. dillenii</i> cactus	PEF	6.6 L/h flow rate, 35 kV/cm electric field strength, and 3 µsec pulse width	Moussa-Ayoub et al. (2017)
<i>O. dillenii</i> cactus	HHP	800 MPa; 25–100 °C and 0.75 L vessel volume	Moussa-Ayoub et al. (2017)
<i>O. ficus-indica</i>	Ultrasound	20 kHz constant frequency with 40%, 60%, 80% amplitude levels for 10, 15, 25 min and pulse durations of 2 sec on and 4 s off.	Reyes-Hernández et al. (2017)
<i>O. ficus-indica</i>	Ultrasound	20 kHz constant frequency with 40%, 60%, 80% amplitude levels and time (10, 15 and 25 min)	Zafra-Rojas et al. (2013)

HHP high hydrostatic pressure, N/A not available, min minutes, PEF pulse electric field, sec seconds

pasteurized and concentrated juices of green cactus pear (*O. ficus-indica*) showed that there was a decrease in lightness ( $L^*$ ) value with thermal treatment, resulting in color losses (Sáenz et al., 1993). The same authors reported that acidification of the *Opuntia* juice and subsequent thermal treatment applied for its microbiological stability and conservation triggered a visual color change. However, the purple-reddish

color of cactus pear fruit juice was stable (Sáenz et al., 1997). Also, the stability of *O. ficus-indica* juice was improved using pasteurization (20 min at 100 °C) but with dramatic effects on the flavor and color sensation. Aside from the conventional thermal processing (sterilization, and pasteurization) method, some of the benefits such as lower capital cost, shorter treatment time, and better energy efficiency were associated with other non-conventional thermal processes (microwave and ohmic heating) (Salazar-Gonzalez et al., 2014; Lee et al., 2015). Their major setbacks were the decline in nutritional, functional and sensory properties of food as well as a reduction in conductive and convective heat transfer rate (Rawson et al., 2011; Iqbal et al., 2019). Therefore, a mild processing technology such as novel nonthermal processing could be useful to maintain the desired nutritional quality, sensory characteristics, and product safety.

According to Iqbal et al. (2019), nonthermal techniques are preservative treatments, effective at ambient temperatures, and applied to lessen undesirable thermal treatments that affect nutritional attributes. These processing technologies are highly sophisticated as compared to thermal processing but very expensive to small-scale processors. Several factors, such as the type of foods, processing conditions and time, processing intensity, and among others, should be thought of before using these technologies (Adebo et al., 2021). Nonthermal technology has been reported as effective preservative techniques that maintain the nutritional and microbiological properties of juice derived from *Opuntia* spp. (Moussa-Ayoub et al., 2011b, 2017; Zafra-Rojas et al., 2013; Cruz-Cansino et al., 2016). One such treatment is the ultrasound (sonication) treatment, an emerging, simple, reliable, environmentally friendly, and cheap technology for achieving effective microbial decontamination (Tiwari et al., 2009b; Zafra-Rojas et al., 2013). The ultrasound technology exerts several effects, such as vibrations or cavitation, heat as well as mass transfer, that can all be useful before conventional food processing and preservation methods such as frying, extrusion, brining, cutting, freezing, homogenization, accelerated fermentation, drying, and others (Chemat & Khan, 2011). Due to the inhibitory effect on enzymatic browning, this technology has gained attention in the fruit industry and already been reported as an alternative to heat treatments in processing fruit juices without compromising their health benefits and nutritional qualities (Cheng et al., 2007; Bhat et al., 2007; Iqbal et al., 2019). Ultrasound technology has also been used to induce microorganisms and enhance the quality of *Opuntia* juices (Zafra-Rojas et al., 2013; Cruz-Cansino et al., 2015, 2016).

HHP has been successfully used in beverages as a versatile industrial approach to inactivate pathogenic microorganisms (Georget et al., 2015). Castro and Saraiva (2005) stated that HHP for fruit juices is particularly useful for the inactivation of microbes, and pressure around 600 MPa is commercially accepted. Regarding the exploitation of HHP for food preservation and high-quality foods, a substantial microbial inactivation in fruit juice was attained at ambient temperatures and pressure of 400 MPa or higher within an appropriate holding time for several minutes (Barba et al., 2012). During PEF treatment, microbial inactivation is achieved at temperatures lower than the point, which adversely affects food quality (Raso & Barbosa-Canovas, 2003). It is complex to ascertain the precise mechanism of

chemical reactions during PEF processing in natural systems such as juice or concentrate and products already available in the market (Tiwari et al., 2009a).

Both PEF and HPP technologies have nevertheless been applied for processing *Opuntia* fruit (García-García et al., 2015; Jiménez-Aguilar et al., 2015). For instance, Moussa-Ayoub et al. (2017) examined the effect of PEF (35 kV/cm, 85 kJ/kg), HHP (600 MPa, 35 °C, 10 min), and thermal treatment (95 °C, 3 min) on the physicochemical, antioxidant compounds and rheological properties as well as microbial inactivation of *O. dillenii* juice. The authors observed a 1-log cycle lower microbial content after applying PEF, HHP, and thermal treatment, while the non-thermal treatments retained ascorbic acid more (95%) compared to 78% retention post thermal treatment. The effects of HHP (400–550 MPa/room temperature/0–16 min) and thermal sterilization on the nutritional content and antioxidant activity in prickly pear beverages were also investigated, with the obtained results showing that HHP will either have or retain its novel effects on the nutritional value in *Opuntia* drinks (Jiménez-Aguilar et al., 2015).

## 5 Advantages of Novel Process/Techniques Over the Traditional Methods

With the recent modernization, novel techniques have commercially become important in food processing over the traditional methods. The advantages of novel food processing techniques have been well documented (Knorr et al., 2009; Stoica et al., 2013; Jambrak et al., 2019; Adebo et al., 2021). They tend to preserve the characteristic properties of food better, together with their nutritional and organoleptic properties compared to the traditional method. Further to this is reducing processing time, which is less heat damage and their ability to decontaminate/degrade food contaminants (Adebo et al., 2021). They also improve the physicochemical properties of foods by minimizing food processing intensities, reduce energy requirements, waste load, and to no small extent increase production and process efficiency. Several documented studies have revealed that novel food processing techniques could improve the qualities of *Opuntia* beverages and/or retained their properties better than conventional processing techniques. However, as Steinhart (2006) documented, many more studies into these innovative techniques, regarding their effectiveness and possibly processing by-products still need to be investigated and optimized for useful application of these novel techniques to juice processing.

## 6 Packaging and Commercialization of *Opuntia* spp. Juices

The packaging is essential in the food and beverage industries. Packaging plays many essential roles in the safety of the juice, prevents oxidation and microbial contamination. A market survey found that most of the cactus pear juice producers sell the juice in packed plastic bottles. Also, cans and tetra packs are the formats of packaging for the juice. Manufacturers are concerned about the shelf life of the prickly pear juice. Some manufacturers produce juice concentrate, syrup, and squash. Consumers can further dilute it to consume. As the fruit juice of *Opuntia* spp. is susceptible to microbial degradation, quality and safe packaging prolong the prickly pear juice's shelf life. Quality packaging is the key factor for the internationalization and commercialization of prickly pear juice. Unlike other juices such as mango juice, pomegranate juice, and orange juice, the juice obtained from prickly pears has not gained much attention from modern consumers. Further, the juice is less popular among the populace than in the semi-arid region where the cactus fruits are grown. As microbial stability of the juice is a major worry for the manufacturers and the retailers, dehydrated juice powder provides an alternative for the entrepreneurs. Rodríguez-Hernandez et al. (2005) optimized the spray drying method to produce a dehydrated cactus fruit powder and further evaluated the reconstituted juice. The authors concluded that the best conditions for the highest vitamin C retention were at 205 °C and 0.1 MPa along with maltodextrin 10 dextrose equivalent. However, the powder with the least moisture content was obtained at 225 °C and 0.2 MPa. Such powders are convenient for packaging and also for the supply chain. However, there was a small change in the appearance and the color of the reconstituted juice compared to the fresh juice. Advances in technologies would lead to the improved restoration of nutrients and organoleptic properties of such dehydrated powders. The stressful lifestyle of modern consumers has changed the thoughts in the selection of foods and beverages. The consumers' preference is more towards the ready to drink or ready to serve foods/beverages. However, certain health claims through proper clinical trials would be helpful for the commercialization of the juice. Certain clinical trials should be conducted to study the hypoglycemic effect, cardioprotective effect, impact on the digestive health, and antioxidant potential of the different cultivars of the cactus fruits *in vitro* and using animal models. Promising and reproducible findings validated by regulatory agencies can be presented on the package to attract health-conscious consumers.

## 7 Microbiological Quality and Safety of Juices

Fruit juices processed under hygienic conditions could significantly enhance consumers' well-being through mitigation of urinary tract infection, breast cancer, and congestive heart failure (Abisso et al., 2018). Though, despite these enhancing health benefits, alarms have been raised over their microbiological quality and



safety, with several studies reporting the incidence of foodborne diseases after consumption of fruit juices (Mosupye & Holy, 2000; Chumber et al., 2007; Khan et al., 2015; Nawawee et al., 2019). According to some studies, the prevalence of unhygienic conditions, improper handling, and contamination of raw materials have contributed significantly to the access of bacteria pathogens in fruit juices (Oliveira et al., 2006; Mahale et al., 2008; Khan et al., 2015). However, contamination with these pathogens is less likely to occur after the fruit juice has been aseptically processed and packed for sale. However, contamination may resurface during the juice's opening and serving due to poor hygiene (Visser et al., 2010). These may lead to health challenges starting from mild abdominal bloating and gas to serious episodes of food poisoning and dehydration, which may eventually result in death. While novel food processing techniques play a huge role in reducing and eliminating microbial load, concerted efforts should be made by consumers in mitigating against juice contamination during consumption.

## 8 Conclusion

Juice processing technology plays a vital role in the commercialization and quality of any processed juice. This chapter elucidates the traditional and modern processing technologies such as ultrasound, microwave heating, pulsed electric fields, and high hydrostatic pressure in the *Opuntia* juice processing. The technologies have been useful in enhancing the microbiological quality and shelf-life of juice. The new packaging processes of the *Opuntia* juice have also been highlighted. The commercialization of the *Opuntia* juice in a global forum is possible only by applying new processing and packaging technologies.

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**Part V**  
**Food and Non-food Applications**  
**of *Opuntia* spp.**

# Chapter 29

## Potential Use of Prickly Pear Juice Prepared from Shelf-Grown Cultivars as an Authentic and Nutritional Fruit Supplement



Vassilios K. Karabagias, Ioannis K. Karabagias, and Anastasia V. Badeka

**Abstract** As the basic discipline of the Mediterranean diet, the regular consumption of fruits and vegetables has been associated with tremendous health benefits for humans and the defense against numerous diseases. Prickly pear is a well type of fruit cultivated in Mexico and the US, in Central and Northern Europe, and numerous Mediterranean countries. It is considered a sort of exceptional pleasure, while it is generally consumed as fresh fruit or used to produce various products such as canned fruits, sorbets, prickly pear juice, jams, dried fruits, drinks from the fermentation of the fruit's pulp. Recent studies have demonstrated the nutritional and functional character of prickly pear or its juice as an antioxidant, anti-carcinogenic, anti-atherosclerotic, anti-ulcerative, hepato-protective, and immune-protective food source, based on its phytochemical (flavonoids, phenolic acids, carotenoids), vitamin C, minerals, free amino acids, and phytosterol contents. The present chapter's objective is to highlight the potential use of prickly pear juice prepared from fruits of the wild cultivars grown in different parts of the Peloponnese Peninsula as an authentic and advanced fruit supplement.

**Keywords** Peloponnese peninsula · Shelf-grown cultivar · Shelf-life · Polyphenols · Antioxidant activity · Cultivar impact

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## 1 Prickly Pear Fruit

There are approximately 300 cactus species of the genus *Opuntia* growing in various parts of the world, mainly in South and East America, Europe, numerous Mediterranean countries, Asia, Africa, and Australia (Madrigal-Santillán et al., 2013). The species of the genus *Opuntia* have the particularity to thrive in arid and semi-arid areas (mountainous and non-mountainous areas) with a low annual rainfall rate. Certain *Opuntia* genus species may be used to produce edible flavoring nuts and numerous products originating either from the fruit or other parts of the plant (Hamdi, 1997; Stintzing et al., 2000). This adjustment may mainly serve as a beneficial and alternative crop for many regions' rural populations, as supported by the Food and Agriculture Organization (FAO) (Stintzing et al., 2000). However, the exploitation of prickly pear products is still limited because these belong to perishable goods (Cruz-Cansino et al., 2016). In the Hellenic zone, *Opuntia ficus indica* (L.) thrives mainly in Peloponnese, Crete, Central, and Western Greece. Some typical cultivated varieties grown in Peloponnese include *Sulfarina*, *Sanguigna*, *Ellisiana*, *Santa Ynez*, *Muscareda Bianco*, *Agave Americana*, which are genetically altered hybrids of the shelf-grown (wild) cultivars (Karabagias et al., 2019a). Large plantations of the species *Opuntia ficus indica* (L.) are harvested in Sicily, where the fruit is an important agricultural product. In Mexico, Chile and Israel numerous crop optimization studies have been carried out to increase the fresh fruit's annual production. At normal plant growth conditions, prickly pear's annual production reaches ca. 3–4 tons per acre (Lee et al., 2000). In Mexico, Peru, and the Mediterranean regions (Italy, Sicily, Malta, Spain, Algeria, and Tunisia) and many tropical countries, prickly pear is cultivated for fruit production. It comprises an essential ingredient in the diet of local inhabitants. In Central and Northern Europe and the US, it is considered exceptional pleasure and is generally consumed as fresh fruit. In the United States, Mexico, and Italy, it has been reported that prickly pear is traditionally used for the production of various products such as canned fruits, sorbets, prickly pear juice, jams, dried fruits, and drinks from the fermentation of the fruit's pulp (El Kossori et al., 1998). Many years ago, the Mexicans used prickly pear leaves for therapeutic purposes (Stintzing et al., 2000).

## 2 Chemical Composition

The chemical composition of the edible part of the prickly pear fruit concerns high water content, and more specifically, 82 g out of 100 g of fruit is water. It also contains glucose and fructose of about 29% and 24%, respectively, which confirms the pulp's sweetness, while sucrose is found in a lesser amount. The fruit pulp is rich in potassium, calcium, magnesium, and phosphorus. More specifically, in the same amount of sample (100 g), potassium covers 220 mg, calcium 56 mg, and magnesium 85 mg, while phosphorus covers 24 mg. It also contains 14 mg of ascorbic acid (vitamin C). Other constituents of prickly pear are protein and fiber-rich in pectin.



**Table 29.1** Chemical composition of prickly pear fruit<sup>a</sup>

<b>Energy</b>	172 kJ (41 kcal)	%DV
<b>Carbohydrates</b>	9.6 g	
Dietary fiber	3.6 g	
Fat	0.5 g	
<b>Protein</b>	0.7 g	
<b>Vitamins</b>		
Vitamin A equivalents	25 µg	3%
Riboflavin (B2)	0.1 mg	8%
Niacin (B3)	0.5 mg	3%
Pyridoxine (B6)	0.1 mg	8%
Folate (B9)	6 µg	2%
Vitamin C	14.0 mg	17%
<b>Minerals</b>		
Calcium	56 mg	6%
Iron	0.3 mg	2%
Magnesium	85 mg	24%
Phosphorous	24 mg	3%
Potassium	220 mg	5%
Zinc	0.1 mg	1%
<b>Other Constituents</b>		
Water	88 g	

Source: USDA Nutrient database, FoodData Central, <https://fdc.nal.usda.gov/>

<sup>a</sup>Nutritional value per 100 g%DV: Percentages of daily value are roughly approximated using the US recommendations

However, the prickly pear composition may vary, depending on the botanical origin of the fruit and numerous environmental factors of the place of cultivation. The juice's color is due to betalains (betacyanins and betaxanthins) (Albano et al., 2015). The fruit also contains flavonoids, such as quercetin and isorhamnetin. According to the United States Department of Agriculture nutrient database (USDA, 2020), its nutrient content is shown in Table 29.1. However, the prickly pear juice of the shelf-grown cultivars grown in the Peloponnese Peninsula may have a different chemical composition concerning water, fat, ash, micro- and macro-minerals contents (Table 29.2).

## 2.1 Physico-Chemical Parameters

The average acidity values of prickly pear juice prepared from the shelf-grown cultivars in the region of Peloponnese Peninsula (0.11–0.15% citric acid) (Karabagias et al., 2019a) were higher compared to those of Moroccan prickly pear juice

**Table 29.2** Chemical composition of prickly pear fruit<sup>a</sup> of shelf-grown cultivars and mineral content of the respective juice<sup>b</sup>

Water (g/100 g)	Fat (g/100 g)	Ash (g/100 g)	Calcium (mg/kg)	Iron (mg/kg)	Magnesium (mg/kg)	Phosphorous (mg/kg)	Potassium (mg/kg)	Zinc (mg/kg)
65.46 ± 3.99–	0.10 ± 0.01–	2.55 ± 0.03–	59.61 ± 20.72–	0.76 ± 0.70–	93.92 ± 11.80–	127.52 ± 17.70–	1869.7 ± 205.64–	0.79 ± 0.22–
69.07 ± 0.59	0.28 ± 0.02	3.29 ± 0.04	89.66 ± 33.85	1.36 ± 2.33	108.50 ± 14.74	188.53 ± 28.94	2398.60 ± 252.36	1.30 ± 0.56

<sup>a</sup>Unpublished data by Karabagias et al. (2019a)<sup>b</sup>Karabagias et al. (2019b)

prepared from different domestic cultivars (0.046–0.098) (Dehbi et al., 2014), but lower than those reported for Egyptian *Algeria* prickly pear juice (0.22% citric acid) (Mohamed et al., 2014). Vitamin C content also recorded significantly ( $p < 0.05$ ) higher values (52.8–55.3 mg/100 mL) compared to Sicilian (31–38 mg 100 g<sup>-1</sup>) (Gurrieri et al., 2000) and Egyptian (17.5 mg/100 g) (Mohamed et al., 2014) prickly pear juices. The effective acidity (pH) values were also differentiated ( $p < 0.05$ ) among prickly pear juice samples of different geographical origin (5.70–6.03) (Karabagias et al., 2019a), but were in general agreement with those pH values involving Sicilian (6.4–6.5) (Gurrieri et al., 2000), Egyptian (6.02) (Mohamed et al., 2014) and Moroccan (5.27–5.95) (Dehbi et al., 2014) prickly pear juices. Considering the pH of prickly pear juice and its low acidity, it can be well consumed by people suffering from stomach problems when they consume acidic goods. Significant variations ( $p < 0.05$ ) were also reported by Karabagias et al. (2019a) for electrical conductivity (EC), salinity content, along with total dissolved solids (TDS) content concerning the geographical origin of fruit cultivars. There is no available data in the literature reporting such values (Gurrieri et al., 2000; Mohamed et al., 2014; Dehbi et al., 2014). Lamsal and Jindal (2014) reported that EC values of orange, pineapple, and tomato fruit juices were affected by fruit geographical origin. The average values ( $\mu\text{S cm}^{-1}$ ) were 3430, 2950, and 5050, significantly higher than those of prickly pear juice. The edible portion of Malay apple fruit (*Syzygium malaccense*) grown in Brazil showed significant differences ( $p < 0.05$ ) in total soluble solids content concerning fruit geographical origin (Nunes et al., 2016). A previous study has reported significantly higher EC (average range of 1890–2270  $\mu\text{S/cm}$ ) and total dissolved content (average range of 936–1140 mg/L) values for freshly prepared orange juice of the Merlin cultivar. In contrast, EC and TDS content values were significantly ( $p < 0.05$ ) affected by orange juice geographical origin (Nikolaou et al., 2017), in agreement with the results of Karabagias et al. (2019a). The total sugar content of fruit juices may be defined as Baumé or Brix degrees. It can be calculated by the specific weight of juice (ICUMSA, 2019) expressed as the % by weight of fruit sugar in solution at a specified temperature of 1.8°Brix = 1°Baumé. Sugar content, along with acidity, may be indicators of the developed flavor and stability of fruit's juices and maturity level. In the case of prickly pear juice samples from the Peloponnese Peninsula, Baume values ranged from 6.00 to 7.44 (Karabagias et al., 2019b), in agreement with the data concerning Egyptian prickly pear juice (10.7°Brix) (Mohamed et al., 2014) or Moroccan prickly pear juice prepared from different cultivars (11.3–15.4°Brix) (Dehbi et al., 2014). The Greek prickly pear juice also recorded a significantly lower total sugar content than other fruit juices such as guava and mandarin (Mohamed et al., 2014) or orange (Nikolaou et al., 2017). Another physico-chemical parameter that is of great importance for consumer developed research is the color of fruits. Concerning prickly pear juice from the Greek regions in the Peloponnese Peninsula, the brightest prickly pear juice samples (highest  $L^*$  values) were those from Eastern Messinia ( $L^* = 48.7$ ), followed by those of Lakonia ( $L^* = 45.1$ ) and Western Messinia ( $L^* = 44.6$ ) regions, respectively (Karabagias et al., 2019a). Reddish ( $a^*$  values) and yellow components ( $b^*$  values) (pigments) were present in all analyzed juice samples of the above

study. In particular, prickly pear juice from the region of Western Messinia had the highest  $a^*$  values (32.3), followed by those of Lakonia (28.2) and Eastern Messinia (23.8) regions. On the other hand,  $b^*$  colour parameter values were significantly ( $p < 0.05$ ) higher in prickly pear juice samples from the region of Eastern Messinia. The respective order was: Eastern Messinia (72.9) > Lakonia (70.8) > Western Messinia (68.8). Chromaticity parameters of Greek prickly pear juices were significantly higher than those reported for Egyptian ones (Mohamed et al., 2014). The possible reasons behind the obtained variations in the physico-chemical parameters of prickly pear juice samples of different geographical origin may include differences in the climatic and soil conditions of the investigated areas, as reflected by the differences in the degree of (1) rainfall, (2) sunlight, (3) temperature, (4) humidity, (5) altitude, and differences in the fruit cultivar (Dehbi et al., 2014), in combination with the micro-differences in the fruit maturity level (Nikolaou et al., 2017).

### 3 Processing Strategies

Nowadays, the uses of prickly pear are diverse. Prickly pear is mainly cultivated for its fruits. In Mexico, in addition to its fruits, it is cultivated for its sprouts, consumed as a vegetable. In the Canary Islands, the prickly pear is cultivated to breed an insect whose scientific name is *Dactylopius coccus* and used to produce a red pigment (cochineal or E120). The insect produces carminic acid, which prevents it from being preyed on by other insects. Carminic acid can be extracted from the body and eggs of the insect to give the red color. This pigment is used in both food and cosmetics. Besides, in the prickly pear, there are two other pigments. More specifically, xanthine (yellow) and betanin (red-violet) are used in the food industry. In Sicily, Ficodi liqueurs are made from prickly pears. Also, a product of particular interest is the gluten-free prickly pear flour. In summary, from prickly pear, they can produce several products such as jams, jellies, syrups, spoon sweets, compotes, and dried prickly pears. Prickly pear fruit can give a high-vitamin C content juice. Also, alcoholic beverages such as tequila, raki, liqueur, spirits, or even beer can be prepared from the fruit/fruit juice by alcoholic fermentation (Karabagias et al., 2020). However, prickly pears are not only applicable to the food industry but also to the pharmaceutical industry. A standard product in high demand is the oil produced from prickly pear seeds and marketed as a youth elixir. It is a vegetable oil rich in vitamins, minerals, polyunsaturated fatty acids, and antioxidants, which act against skin aging. In Mexican medical practice, prickly pear juice is considered a beneficial treatment for the digestive and urinary tract wounds and inflammation. Due to its flavonoids, it is potent for the immune system, while its flowers and leaves are used as diuretics, antispasmodics, hemolytic, and the defense against nephritis (Kim et al., 2015). The medicinal value of prickly pear is excellent as it is rich in magnesium, and the amino acid taurine, which is essential for the brain and heart's health. It is also rich in flavonoids and antioxidants that "work" against cancer and heart disease. Besides, it is rich in vitamin B6, which helps maintain low levels of

homocysteine, the main component that accumulates in vessels and increases the risk of heart attack and stroke. Consuming foods high in beta-carotene and beta-cryptoxanthin may reduce the risk of developing lung cancer. In conclusion, the multitude of prickly pear nutrients helps to cope with the symptoms of drunkenness, inflammation and trauma, high cholesterol levels, obesity, diabetes mellitus, prostate hypertrophy, and lung disease.

## 4 Stability of Prickly Pear Juice

The perspectives of preparation of a functional, unique, and low-cost fruit drink may be of great interest to the food industry. Nowadays, consumers who are the final judges of a product's acceptance, demand novel, and bio-functional products for their daily diet with potential health benefits (Nunes et al., 2016). Prickly pear juice may comprise the basis of a new commercialized product as it showed a high *in vitro* bio-functionality, low total sugar content, considerable vitamin C content, low sodium content along with specific sensory properties (Karabagias et al., 2019a, b). However, it should not be forgotten that prickly pear juice has shown important biological effects (Galati et al., 2003; Chang et al., 2008; Jiménez-Aguilar et al., 2014). Given that prickly pear fruit belongs to perishable products, the application of the most effective packaging system may aid in a higher shelf-life of prickly pear juice and, of course, is a challenging issue for the food industry. Therefore, a prospective industrial application of prickly pear juice in the international markets, covering the aforementioned issues, may increase the food industry's profits and support the welfare of local economies, as the specific place of origin of a product may affect its price, support, and acceptance. A recent study demonstrated that the shelf-life of prickly pear juice was low in the air packaging material used. Total viable count (TVC) results showed that the shelf life in air packaging was estimated to be 2 days since TVC in juice reached the upper limit of  $7 \log \text{CFU g}^{-1}$  (Riganakos et al., 2017) at Day 2 of storage under refrigeration. Psychrotrophs at Day 2–3 of storage reached the value of  $7 \log \text{CFU g}^{-1}$  (6.78), whereas at Day 8 of storage exceeded this limit (8.60). Better results were obtained for other spoilage bacteria, such as *Enterobacteriaceae* and yeast and molds, where a lower trend in developing such microorganisms was observed and never reached the upper limit of  $7 \log \text{CFU g}^{-1}$ .

On the contrary, vacuum packaging proved to be a favorable technology for the preservation and shelf-life extension of prickly pear juice since TVC ( $6.78 \log \text{CFU g}^{-1}$ ) did not exceed the value of  $7 \log \text{CFU g}^{-1}$  during the 8 days of storage under refrigeration. Besides, there was observed a complete inhibition of psychrotrophs growth under vacuum packaging. Finally, the populations in *Enterobacteriaceae* and yeast and molds remained at lower levels than  $7 \log \text{CFU g}^{-1}$  (5.74 and 6.26, respectively). Based on the microbiological analysis, vacuum packaging extended the shelf-life of prickly pear juice by 7 days, setting the potential of prospective market distribution of such a perishable product.

## 5 Sensory Properties

Consumers are the final judges of the product's fate and welfare in the market as their preference is of vital significance. Therefore, a product's specific sensory properties, along with its composition, may comprise a key for its uniqueness and global support. Concerning the unprocessed prickly pear juice prepared from shelf-grown cultivars possessed a fruity, grass, pear, cucumber, peach, or pear-like flavor as mentioned by the panelists in the study of Karabagias et al. (2019a). A semi-sugary, slightly acidic taste and a bright red to orange color with yellow and green notes were also reported. The descriptive test analysis (Sidel & Stone, 1993) results showed that prickly pear juice's shelf-life was 3 days for the air packaging and 8 days for the vacuum packaging. This is not the first time in the literature in which the microbiological (see the section above) and sensory data conflict. The total mesophilic micro-flora may increase value; however, all the microorganisms cannot cause spoilage (Jay, 1986). As an overall estimation, prickly pear juice was a very acceptable fruit drink, resulting as a "freshener". This finding agrees with the results of Mohamed et al. (2014). They reported the high acceptance of Egyptian prickly pear juice among mandarin or guava juices, during the sensory examination, in terms of color, odor, taste, mouthfeel, and appearance.

## 6 Antioxidant Activity and Total Phenolic Content

### 6.1 In Vitro Antioxidant Activity

Prickly pear fruit and its juice are products of high *in vitro* antioxidant activity and total phenolic content, indicating a strong potential to be a regular fruit supplement (Chavez-Santoscoy et al., 2009). Indeed, in the study of Karabagias et al. (2019a), prickly pear juice samples showed a considerable *in vitro* antioxidant activity. The most pronounced results were recorded for Eastern Messinia prickly pear juice samples (75.6%) (yellow to green cultivar) followed by those of Lakonia (69.9%) (red to orange cultivar), and Western Messinia (67.3%) (yellow to green cultivar), respectively. This sequence reveals differences in the presence/and or content of specific bio-molecules (carotenoids, polyphenols, proteins, and vitamin C) that possess antioxidant activity based on prickly pear juice geographical origin, apart from the impact of the cultivar. Cruz-Cansino et al. (2016) reported that Mexican prickly pear juice prepared from two (red and green) domestic prickly pear cultivars had antioxidant activity values ranging between 58.9% and 66.9%. However, an exhaustive *in vivo* mechanism of how antioxidants react against free radicals is still scarce. A previous study reported that the consumption of antioxidants could reduce oxidative stress in an organism (Arena et al., 2001). The preventive administration of Sicilian prickly pear juice inhibited ethanol's ulcerogenic activity in rats (Galati et al., 2003). It was shown that prickly pear juices prepared from three different

Mexican cultivars (red-purple, white-green, and yellow-orange) recorded a considerable antioxidant activity using the DPPH· assay, whereas showed an anticlastogenic potential against methyl methanesulfonate (MMS), given as feed-in mice (Madrigal-Santillán et al., 2013). The antioxidant potential of a cocktail of fruits or a specific fruit may be defined not only by a specific substance/molecule, but it may arise from the sum of the antioxidant capacity of each of its components. Thus, the beneficial components present in prickly pear fruit can interact with each other, when consumed, to give synergistic effects (Satué-Gracia et al., 1997). The antioxidant activity of prickly pear juice is probably attributed to a synergistic antioxidant mechanism between betalains, flavonoids, or other phytochemicals combined with catalytic minerals, as these compounds comprise the main components (Madrigal-Santillán et al., 2013; Bouzoubaâ et al., 2016; Karabagias et al., 2019b).

## 6.2 Total Phenolic Content

Numerous micro-nutrients and phytochemical compounds such as betaxanthins (yellow-orange) and betacyanins (red-violet) (Zafra-Rojas et al., 2013), along with the flavonoids quercetin, isorhamnetin, kaempferol, and luteolin (Kuti, 2004; Fernández-López et al., 2010) and phenolic acids, caffeic acid, ferulic acid, *para*-coumaric acid, and sinapinic acid (Ndhlala et al., 2007; Chang et al., 2008) have been reported to enrich the composition of prickly pear fruit. Working with prickly pear fruits of the shelf-grown cultivars in the Peloponnese Peninsula, Karabagias et al. (2019a) reported that prickly pear juice samples from the area of Eastern Messinia had a significantly higher total phenolic content ( $p < 0.05$ ) followed by those of Lakonia and Western Messinia regions. The respective values were  $7592.1 \pm 2441.0$ ,  $3284.8 \pm 906.3$ , and  $3234.5 \pm 978.2$  mg GAE/L. As can be observed, this is a considerably high total phenolic content. What is also remarkable is the fact that the prepared juice from prickly pear fruits harvested in Eastern and Western Messinia possessed a significantly different total phenolic content ( $p < 0.05$ ), indicating the great impact of geographical origin on fruit composition and properties. Total phenolic content of prickly pear juice from the regions of Lakonia and Western Messinia was 5-times higher than those reported in previous works involving Moroccan (Dehbi et al., 2014) and Mexican (Cruz-Cansino et al., 2016) prickly pear juices, whereas that of Eastern Messinia was *ca.* 12-times higher. Galati et al. (2003) reported the presence of ferulic acid (among other phenolics) in Sicilian prickly pear juice in high amounts ( $746 \mu\text{g mL}^{-1}$ ). To highlight the significance of their findings, the authors characterized it as the “chief phenolic derivative”. Abdel-Hameed et al. (2014) reported that the total phenolic content of prickly pear juice prepared from two domestic cultivars grown in Saudi Arabia ranged between 6678 and 11,529 mg GAE/L. However, the content of phenolic compounds in prickly pear is variable, depending on genetic and environmental factors, and it is probably related to the color of the fruit (see Sect. 6). It has been reported that the purple prickly pear pulp and the red ones contain the highest total phenolic content,

followed by orange and finally green varieties (Stintzing et al., 2005). An additional parameter that could explain the differences in prickly pear fruits' total phenolic content and their products is the extraction solvent used. In the study of Karabagias et al. (2019a), the water-soluble antioxidants were taken into account based on the fact that fruit juice is an aqueous solution of fruit matrix and only in this form may be consumed by humans. Concerning the impact of geographical origin on prickly pear juice antioxidant properties and total phenolic content, Karabagias et al. (2019a) applied mathematical correlations. There was a perfect Spearman's correlation ( $\rho = 1.00$ ) between the average values of *in vitro* antioxidant activity and total phenolic content of juice samples, concerning geographical origin ( $p = 0.01$ ). Spearman's correlation is a **non-parametric** measure of **rank correlation** (**statistical dependence** between the **rankings** of two **variables**). It assesses how well the relationship between two variables can be described using a **monotonic** function (Corder & Foreman, 2009).

## 7 Authentication Issues and the Potential of Prickly Pear Juice as a Special Fruit Supplement

The ability to accurately determine the botanical and geographical origin of food-stuffs by highlighting specific physicochemical and bio-functional properties these possess is of great importance for the market distribution, price, research, and acceptance of product (El Kossori et al., 1998; Nikolaou et al., 2017). The primary tool for analysts to indicate these differences is chemometrics. This section includes a case study concerning the cultivar differentiation of prickly pear juice based on the use of physicochemical and bio-activity parameters. The data comprise unpublished material of the previous study (Karabagias et al., 2019a).

### 7.1 Statistical Analysis

As a pre-evaluation procedure, multivariate analysis of variance (MANOVA) was applied to all physicochemical and bio-activity parameter values to highlight those significant variables that could differentiate prickly pear juice samples according to cultivar. In MANOVA analysis, only the average values of a series of analyzed samples are used. Wilks Lambda index, as a probability distribution used in multivariate hypothesis testing, was computed to determine a possible significant effect of the examined parameters concerning the cultivar of prickly pear juice samples. Linear discriminant analysis, as a supervised recognition technique, was then applied to generate the classification of a group of subjects/variables. The linear combination of the defined by MANOVA significant parameters ( $p < 0.05$ ) results in the formation of a discriminant function ( $Y$ ) of the specific form:



$$Y = a_1X_1 + a_2X_2 + \dots + a_nX_n, \quad (29.1)$$

where  $a_1, a_2, \dots, a_n$  are the standardized canonical discriminant function coefficients and  $X_1, X_2, \dots, X_n$  are the independent variables. Both the original and cross-validation methods were considered to provide the correct prediction rates (Miller & Miller, 2010).

## 7.2 *Cultivar Recognition of Prickly Pear Juice Based on Physicochemical and Bio-activity Parameter Values Complemented with Chemometrics*

Multivariate analysis of variance (MANOVA) and linear discriminant analysis (LDA) was applied to the whole set of data (Table 29.3). The data were grouped according to cultivar: Yellow to green fruits (Cultivar I) and red to orange fruits (Cultivar II). The indices of multivariate hypothesis Pillai's Trace = 0.834 ( $F = 13.095$ ,  $df = 13$ ,  $p = 0.000$ ) and Wilks' Lambda = 0.166 ( $F = 13.095$ ,  $df = 13$ ,  $p = 0.000$ ) showed that there was a significant impact of the cultivar (yellow to green, and red to orange) on the measured physicochemical and bio-activity parameter values. In particular, eight physicochemical and bio-activity parameters were significant for the cultivar distinction of prickly pear juice (Table 29.4).

Even though total dissolved solids (TDS) content was significant ( $p = 0.028$ ) in differentiating the prickly pear juice samples according to cultivar, it did not pass the tolerance test during the LDA analysis (Table 29.5).

Tolerance is the proportion of a variable's variance not accounted for by other independent variables in the discriminant function developed. A variable with very low tolerance contributes little information to a predictive model and may cause computational problems (Karabagias et al., 2019b). Discriminant analysis showed that one statistically significant discriminant function was formed: Wilks' Lambda = 0.239, Chi-square ( $X^2$ ) = 60.774,  $df = 7$ ,  $p = 0.000 < 0.001$ . The canonical discriminant function had an eigenvalue of 3.179 and a correlation of 0.872, accounting for 100% of the total variance. The unstandardized canonical discriminant functions evaluated at group means are represented by the functions at group centroids. Each centroid has two numbers, which represent the coordinates. The abscissa is the first discriminant function, and the ordinate is the second (Karabagias et al., 2019b). Given that in our case 1 discriminant function was formed (only two groups of factor variables), the respective group centroid values were:  $-1.413$  for yellow to green cultivar (Cultivar I), and  $2.156$  for the red to orange cultivar (Cultivar II). The Fisher's linear discriminant functions representing the two cultivars' algorithms are of the following form (Eqs. 29.2 and 29.3), considering the classification function coefficients in Table 29.6.

**Table 29.3** Multivariate analysis of variance (MANOVA) and linear discriminant analysis (LDA) applied to the whole set of data

Cultivar	Acidity (g/100 mL)	Vitamin C (mg/100 mL)	pH	EC ( $\mu$ S/cm)	Salinity (g/L)	TDS (mg/L)	Specific weight (g/mL)	Baumé $L^*$	$a^*$	$b^*$	Antioxidant activity (%)	TPC (mg GAE/L)
Yellow to green												
Average (N = 29)	0.14	53.55	5.85	580.97	0.31	291	1.05	6.57	47.20	27.17	71.36	5939
$\pm$ SD	0.03	3.93	0.38	55.38	0.03	28	0.00	0.56	3.13	4.67	4.20	2931
Red to orange												
Average (N = 19)	0.12	55.36	6.03	613	0.33	307	1.05	7.44	45.08	28.20	70.80	3285
$\pm$ SD	0.01	6.17	0.13	31	0.02	15	0.00	0.32	2.45	3.66	3.45	906

*N* number of samples, *EC* electrical conductivity, *TDS* total dissolved solids, *TPC* total phenolic content, *GAE* gallic acid equivalents

**Table 29.4** Significant ( $p < 0.05$ ) physico-chemical and bio-activity parameters for the differentiation of prickly pear juice according to cultivar

Tests of equality of group means					
Physicochemical and bio-activity parameters	Wilks' Lambda	F	df1	df2	Sig. ( $p$ )
Acidity	0.851	8.055	1	46	0.007
Vitamin C	0.967	1.554	1	46	0.219
pH	0.921	3.953	1	46	0.053
Electrical conductivity	0.898	5.220	1	46	0.027
Salinity	0.825	9.781	1	46	0.003
Total dissolved solids	0.899	5.172	1	46	0.028
Specific weight	0.535	39.972	1	46	0.000
Baumé	0.551	37.433	1	46	0.000
$L^*$	0.881	6.219	1	46	0.016
$a^*$	0.982	.0865	1	46	0.357
$b^*$	0.995	0.236	1	46	0.630
Antioxidant activity	0.969	1.485	1	46	0.229
Total phenolic content	0.759	14.573	1	46	0.000

$df$  degrees of freedom,  $F$  values of the Fisher's distribution,  $p$  probability

**Table 29.5** Variables failing tolerance test<sup>a</sup>

Variable	Within-groups variance	Tolerance	Minimum tolerance
TDS	561.779	0.000	0.000

All variables passing the tolerance criteria are entered simultaneously

<sup>a</sup>Minimum tolerance level is .001

Yellow to green cultivar =  $-12,485,535.785 - 23,286.027$  Acidity  $-168.257$   
 Electrical conductivity  $+412,061.001$  Salinity  $+24,962,855.575$  Specific weight (29.2)  
 $-187,782.109$  Baumé  $+711.908L^* + 0.105$  Total phenolic content

Red to orange cultivar =  $-12,490,707.770 - 23,339.074$  Acidity  $-168.378$   
 Electrical conductivity  $+412,349.224$  Salinity  $+24,967,976.370$  Specific weight (29.3)  
 $-187,813.499$  Baumé  $+711.982L^* + 0.104$  Total phenolic content

Table 29.7 shows the allocation of samples according to a group of the cultivar. As observed, of the 29 samples belonging to the yellow to green cultivar, 28 were allocated correctly to their initial group. In contrast, only one prickly pear juice sample was allocated to the red to orange cultivar. Similarly, of the 19 samples belonging to the red to orange cultivar, 18 were allocated correctly to their initial group. In contrast, only one prickly pear juice sample was allocated to the yellow to green cultivar. The overall correct classification rate was 95.8% for the original and 91.7% for the cross-validation method.

**Table 29.6** Classification functions coefficients of the obtained Fisher's linear discriminant functions during the LDA analysis

Classification function coefficients		
Physicochemical and bio-activity parameters	Cultivar	
	Yellow to green (I)	Red to orange (II)
Acidity	-23,286.027	-23,339.074
Electrical conductivity	-168.257	-168.378
Salinity	412,061.001	412,349.224
Specific weight	24,962,855.575	24,967,976.370
Baumé	-187,782.109	-187,813.499
<i>L</i> *	711.908	711.982
Total phenolic content	.105	.104
(Constant)	-12,485,535.785	-12,490,707.770

Fisher's linear discriminant functions

**Table 29.7** Cultivar differentiation of prickly pear juice based on eight physico-chemical and bio-activity parameters using LDA

LDA	Classification rate	Cultivar	Predicted group membership		Total
			Yellow to green	Red to orange	
Method	%				
Original <sup>a</sup>	Count	Yellow to green	28	1	29
		Red to orange	1	18	19
	%	Yellow to green	96.6	3.4	100.0
		Red to orange	5.3	94.7	100.0
Cross-validated <sup>b, c</sup>	Count	Yellow to green	27	2	29
		Red to orange	2	17	19
	%	Yellow to green	93.1	6.9	100.0
		Red to orange	10.5	89.5	100.0

<sup>a</sup>95.8% of original method grouped cases correctly classified

<sup>b</sup>Cross-validation is done only for those cases in the analysis. In cross-validation, each case is classified by the functions derived from all cases other than that particular case

<sup>c</sup>91.7% of cross-validated grouped cases correctly classified

## 8 Conclusions

The systematic and improved cultivation of prickly pear fruits globally is really a challenge and probably a beneficial/profitable crop. Prickly pear juice is a rich source of phytochemicals with a high *in vitro* antioxidant activity. Vacuum packaging may be the leading packaging system in the food industry, given that it extended the shelf-life of prickly pear juice. Based primarily on sensory and secondly on microbiological analysis data shown in the study of Karabagias et al. (2019a), the shelf-life of prickly pear juice prepared from shelf-grown cultivars was 3 days for the air and 8 days for the vacuum packaging, respectively. Finally, both the

geographical and botanical origin of prickly pear juice proved to be the critical factors regarding its physicochemical/bio-activity parameter values and sensory characteristics concerning its authenticity.

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# Chapter 30

## Fermented Beverages from *Opuntia* Species: Composition, Commercialization and Future Outlook



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**Abstract** Beverages play a vital role in the livelihood of human beings. Drinks, particularly those processed from fruits, provide nutritional and health benefits, and they also support socio-economic livelihood. Beverages have also become part of the culture and tradition among indigenous communities, being served at social gatherings, funerals, and marriages. The manufacturing processes of fermented beverages relies on age-long traditional procedures modernized with available equipment and novel processing operations. Development in science and technology has led to the conception of a variety of fermented products like cider, beer, *kombucha*, and wine. Although tropical and cactus fruits have been harnessed for the development of fermented beverages, these products are yet to catch global consumers'

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attention. This chapter provides an overview on the different types of fermented beverages produced from *Opuntia* spp. with information on the composition of these beverages. Additionally, an insight into the status and scope of commercialization of beverages from *Opuntia* spp. as well as projections and future development areas were highlighted.

**Keywords** *Opuntia* beverages · Colonche · Fermented products · Beverage composition · Functional drinks

## 1 Introduction

The increasing need and requirement of the ever-growing populace have led to the development of food that meet nutritional requirements and equally supply health-promoting constituents. *Opuntia* spp. commonly called ‘prickly pear’ is a fruit consumed directly after basic processing operations such as cutting, washing, and decoction (Ramírez-Moreno et al., 2013). It is used in salad preparation, quality enhancers in flour (Kim et al., 2012; Ramírez-Moreno et al., 2015), seed oil extraction (Baccouche et al., 2013), and in the production of a particular type of honey (Mezzour, 2000).

Beverages (fermented and non-fermented) are an essential part of human lives and are produced from various substrates. The manufacturing processes of fermented beverages rest on age-long traditional procedures modernised with microbiological technology and novel processing operations. Development in science and technology of fermentation has led to fermented products on commercial shelves, notably cider, beer, *kombucha*, and wine (Kebede et al., 2002; Adebo, 2020). Beverages have indeed being part of the culture and tradition among indigenous communities, being served at social gatherings, funerals, and marriages.

Recent studies have indicated potential utilizations of *Opuntia* spp. as functional ingredients for soft drinks (Kim et al., 2013; Tsegay & Lemma, 2020). This could have been stimulated by the increasing utilization of *Opuntia* fruit and derived products as functional ingredients, concentrates, food colorants, and beverages (Di Cagno et al., 2016; Tsegay & Lemma, 2020; Castellar et al., 2008). For beverages, several food processes are utilized in transforming *Opuntia* spp. into such derived products. Fermentation is appraised and discussed in this chapter particularly forms of such beverages, their nutritional, physicochemical, and health-promoting properties. Besides, potential developments for fermented beverages from *Opuntia* spp. were also highlighted.

## 2 Beverages from *Opuntia* Species

Different types of beverages have been developed from the *Opuntia* spp. Some are fermented, while others are non-fermented. Nowadays, fruit pulp of *Opuntia* spp. is fermented using specific microorganisms for the formulation of beverages of desired attributes. The juice or the non-fermented beverage is discussed in a separate chapter of this book; hence, it has not been elaborated in this section in detail. Many authors have depicted the biochemical property of the juice. They have raised concerns about the juice's poor stability due to its higher pH (5.3–6.2) compared to other fruit juices such as lemon and grape juices (Yahia & Sáenz, 2011). The juice is often processed into several beverages, primarily through fermentation technology, for a more extended shelf-life period.

### 2.1 Alcoholic Fermentation

Fermented and distilled alcoholic beverages are produced from the fruits of *Opuntia* spp. Traditional fermented alcoholic beverages are produced in Mexico by using the fruits of *Opuntia* spp.

#### 2.1.1 Colonche

Colonche is a wine type of beverage produced by spontaneous fermentation of the fruit pulp of *Opuntia* spp. The fruits are plucked and collected to the households, followed by peeling and stocking the peeled fruits in a bucket outdoors to accumulate microorganisms from the environment for the fermentation. The fruits are crushed in hands (washed for safety) and packed in an earthen pot covered with a thin clean cloth. Once the fermentation is complete, the seeds are sieved out, and the freshly prepared colonche is served, or it is blended with alcohol or juices of other fruits or spices and stored to be served in the name “vino de tuna”. In some parts of Mexico like Mexquitic de Carmona, the juice of the fruit pulp of *Opuntia* spp. are boiled several times before fermentation takes place. Common spices such as cinnamon are also boiled with the juice to improve the final product's organoleptic property. In this process, *pulque* is used as the source of microorganisms and the starter. *Pulque* is another milky white alcoholic beverage native to Central Mexico, prepared by fermenting the agave plant's sap. Some of the colonche producers apply previously produced colonche as a starter for conducting the fermentation process (Ojeda-Linares et al., 2020). The preparation of colonche in two different indigenous processes in Mexico is shown in Fig. 30.1. Similarly, aguardiente is a distilled alcoholic beverage produced in Mexico from *Opuntia xocconostle*'s fruit pulp (Yahia & Sáenz, 2011).

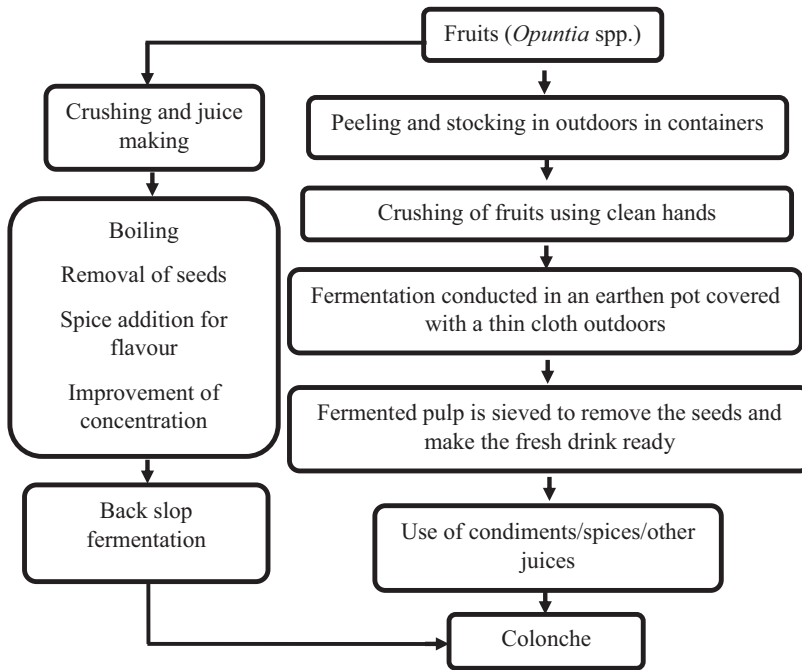


Fig. 30.1 Different processes used for the preparation of Colonche

### 2.1.2 Wine

Lee et al. (2000) demonstrated an alcoholic beverage production using prickly pears juice. It was observed that alcoholic fermentation of 25% prickly pears juice with 22°Brix of sugar with *Saccharomyces cerevisiae* was not successful in preparing the beverage. However, the blending of grape juice with the prickly pear juice in a ratio of 1:1 and fermentation at 22 °C for 6 days could generate an acceptable alcoholic beverage with natural red color. The acceptance of the grape juice blended beverage may be attributed to the lower acidity, anthocyanin content, and the beverage's viscous property. Another combination of fruit blend, *i.e.*, cactus pear fruit juice mixed with *Lantana camara* fruit juice, has been used to make wine, and the fermentation parameters have been optimized (Tsegay & Lemma, 2020). The optimal conditions for the fermentation of cactus pear wine were: 24.8 °C, *L. camara* fruit juice concentration of 10.6% (v/v) and *S. cerevisiae* concentration of 10.1% (v/v), to obtain the wine with an ethanol concentration of 9.53% (v/v) and acceptable sensory property. Rodríguez-Lerma et al. (2011) prepared an alcoholic beverage from prickly pears by fermenting with coculture of *Pichia fermentans* and *S. cerevisiae*. The beverage had an alcoholic content of 8.37% (v/v). The generation of specific volatile compounds responsible for the fruity and ethereal aroma and flavor responsible for the typical wine aroma and flavor was detected. Tsegay et al. (2018) optimized the wine preparation from cactus fruit using response surface methodology and

reported that wine with an alcohol content of 9% and sensory acceptance of 7.74 could be produced at a temperature, 30 °C, pH, 3.9 and inoculum size of 16%. Another interesting study was recently conducted by Karabagias et al. (2020) in which different alcoholic bio-functional beverages such as beer, wine, and whiskey were simulated by using the ethanol extracts of prickly pears juice with different concentrations of ethanol. *In vitro* antioxidant activities for the simulated beer (5% ethanol), wine (12% ethanol), and whiskey (40% ethanol) were studied, and it was observed that the simulated beverages showed higher antioxidant potential (64%) and phenolic contents (7.6 mg gallic acid equivalent/mL) than the juice. Márquez-Lemus et al. (2019) have studied the ethanolic liquor preparation by macerating the fruit pulp with portable ethanol followed by filtration and subsequent addition of inverted sucrose syrup and stored in a bottle. Twenty nine (29) volatile compounds were identified, wherein quercetin was the dominant phenolic compound.

## 2.2 Non-alcoholic Beverages

Non-alcoholic beverages from *Opuntia* spp. include juice, vinegar, and lactic acid fermented juice. Several researchers have developed lactic acid fermented products from cactus pear and elucidated their improved quality and health-promoting properties. Filannino et al. (2016) observed the satisfactory growth of different *Lactobacillus plantarum*, *L. brevis*, *L. rossiae*, and *Pediococcus pentosaceus*. The authors reported that the pulp's fermentation with *L. brevis* POM2 and POM4 resulted in a higher accumulation of  $\gamma$ -amino butyric acid (GABA). GABA is useful in modulating neuronal disorders, anxiety, and improves immunity. The fermented pulp exhibited higher antioxidant potential and improved inhibition of IL-8, prostaglandins PGE2, and TNF $\alpha$  synthesis in contrast to the fresh pulp. An interesting study was conducted by Veron et al. (2019) in which cactus pear fruit juice was fermented using a novel probiotic bacteria *Lactobacillus plantarum* S-811. The health potential was studied in the *S. cerevisiae* model and obese mice. It is worth noting that the lactic acid fermented juice could exhibit 11 times more protection to the yeast cells against 4 mM H<sub>2</sub>O<sub>2</sub> compared to the control (without fermented juice). The fermented juice was also useful in reducing the body weight, hyperglycemia, and hyperlipemia in the aforesaid mice model. Fermentation with lactic acid bacteria is conducted to remove some anti-nutrients and disintegrate risky compounds. A probiotic beverage was optimized using different combinations of prickly pear pulp with permeate, a byproduct of the cheese industry and *Bifidobacterium bifidum*. The organoleptic results revealed that the beverage prepared using 30% prickly pear pulp + 70% permeate + *B. bifidum* (1 g/100 mL) was regarded as the best combination for the preparation of the beverage (Jambi, 2017).

Vinegar is produced from prickly pear, which is available in Mexico, Chile, and Argentina. Pérez et al. (1999) have demonstrated the preparation of yellow-colored vinegar with refreshing aroma by the alcoholic fermentation of sugar fortified cactus fruit juice with 22°Brix, followed by acetic acid fermentation. Similarly, a

balsamic type of vinegar was produced by sequential fermentation of cactus fruit juice with *S. cerevisiae* and acetic acid bacteria to obtain an acetic acid concentration of 35–43 g/L (Prieto et al., 2009). The cactus fruit's pulp and juice exhibit different colors such as orange, yellow, green, and purple; vinegar of various colors were produced. The green-colored vinegar is less preferred to the orange and purple types. *Kombucha* is a refreshing beverage prepared by fermentation of tea with sugar. An innovative beverage was prepared using cactus pear juice as a substrate for the preparation of *kombucha*. The traditional starter culture application resulted in a reduction of pH (5.1–3.5) and an increase in phenol content (23%). The beverage also showed higher antioxidant and antimicrobial activity against pathogens including both gram-negative and gram-positive (Ayed & Hamdi, 2015).

### 3 Characteristics and Composition of Fermented Beverages from *Opuntia* Specie

*Opuntia* spp. fruit, also known as prickly pear, *fico d'india*, tuna, paddle cactus, *nopal*, or *sabra*, is an oval, elongated berry. They exist in a broad spectrum of colors, including orange, yellow, red, purple, and white, relative to their betalain levels (Stintzing & Carle, 2005). While much of the composition of the fruits and plants parts have been discussed in several chapters of this book, focus on the composition of derived fermented beverages are highlighted in this section.

Fermentation has been reported to modify the physicochemical properties and composition of *Opuntia* spp. (Barba et al., 2020). Fermented prickly pear extract (PPE), obtained with *L. rhamnosus* LS, *L. bulgaricus* KCTC3188, and *L. brevis* KCTC3498 was observed to have pH and titratable acidity (TTA) values of 2.90–3.48 and 0.20–1.46%, respectively (Son & Lee, 2004). The authors characterized the beverage as having an attractive red/pink color, with the viability of lactic acid bacteria (LAB) maintained during storage. Fermentation of *O. stricta* juice, particularly for an increase in betanins content, was observed to have yielded a final product with a pH, total soluble content (TSS), betanin, viscosity and density of 3.41, 5.2°Brix, 9.65 g/L, 52.5 cP and 1.17 g/mL, respectively (Castellar et al., 2008). The authors reported that fermentation removed carbohydrates, with bio-alcohol by-product along with concentrated betanin (Castellar et al., 2008). *O. ficus-indica* puree fermented with different starter cultures of *Leuconostoc mesenteroides* was also reported to have ascorbic acid (vitamin C) levels of 13–21 mg/100 mL, pH of 3.72–4.08, TTA of 13.2–17.8%, and viscosity of 4.68–17.9 mPA (Di Cagno et al., 2016). Pigments group of betalains were also reported and quantified with betacyanin and betaxanthine levels of 139.5–170.3 and 69.2–84.3 mg/kg (Table 30.1).

Randazzo et al. (2016), in their study on exploring the use of some Mediterranean fruit juices, developed a fruit-based *kefir* beverage from *O. ficus-indica*. A 2.31% alcohol level was reported, CO<sub>2</sub> of 1.88 g/100 mL, and TSS of 9.67°Brix. The pH, acetic acid, lactic acid, and TTA of the developed beverage were 4.11, 0.16 g/L,

**Table 30.1** Composition of some fermented beverages from *Opuntia* spp.

Parameter	<i>O. stricta</i> juice	<i>O. ficus-indica</i> wine	<i>Opuntia</i> wine	<i>O. ficus indica</i> alcoholic beverage	<i>Opuntia</i> juice	Colonche	Fermented PPE	<i>O. ficus-indica</i> puree	<i>O. ficus-indica</i> beverage
<i>a</i> *	–	–	–	–	–	–	–	6.36–8.42	10.57
Acetic acid (g/L)	–	–	–	–	–	–	–	–	0.16
Alcohol (% v/v)	–	7.65–7.78	–	–	–	8.37	–	–	2.31
Ascorbic acid (mg/100 mL)	–	–	–	–	6	–	–	13–21	–
<i>b</i> *	–	–	–	–	–	–	–	0.96–1.58	16.51
Betamin (g/L)	9.65	–	–	–	–	–	–	–	–
Betacyanins (mg/kg)	–	–	–	–	–	–	–	139.5–170.3	–
Betaxanthines (mg/kg)	–	–	–	–	–	–	–	69.2–84.3	–
CO <sub>2</sub> (g/100 mL)	–	–	–	–	–	–	–	–	1.88
Colour OD@590 nm	10.8 <sup>a</sup>	–	0.17–0.23	–	–	–	–	–	–
Density (g/mL)	1.17	–	–	–	–	–	–	–	–
DPPH (µMTE/mL)	–	–	–	–	105	–	–	–	59.65 <sup>b</sup>
ΔE	–	–	–	–	–	–	–	–	14.91
H <sup>o</sup>	–	–	–	–	–	–	–	–	55.34
Higher alcohols (mg/L)	–	–	200–343	–	–	–	–	–	–
<i>L</i> *	–	–	–	–	–	–	–	20.13–21.02	32.93

(continued)

Table 30.1 (continued)

Parameter	<i>O. stricta</i> juice	<i>O. ficus-indica</i> wine	<i>Opuntia</i> wine	<i>O. ficus indica</i> alcoholic beverage	<i>Opuntia</i> juice	Colonche	Fermented PPE	<i>O. ficus-indica</i> puree	<i>O. ficus-indica</i> beverage
Lactic acid (mg/100 mL)	–	–	–	–	0.32	–	–	–	1.00 <sup>e</sup>
Moisture	50.3	–	–	–	–	–	–	–	–
pH	3.40	–	3.7–4.0	–	4.1	–	2.90–3.48	3.72–4.08	4.11
Reducing sugar (g/100 mL)	–	–	–	–	0.20	–	–	–	–
Residual sugars	–	–	2.0–2.4	–	–	–	–	–	–
Tannins (% w/v)	–	–	0.01–0.07	–	–	–	–	–	–
Tartaric acid (g/L)	–	10.60	–	–	1.7 <sup>d</sup>	–	–	–	–
Titratable acidity (% v/v)	–	–	0.6–0.74	–	–	–	0.20–1.46	13.2–17.8	1.92 <sup>c</sup>
Total antioxidant (mg AA/E/L)	–	203.44	–	–	–	–	–	–	–
Total esters (mg/L)	–	–	20–35	–	–	–	–	–	–
Total phenol (mg/L)	–	444.39	–	–	–	–	–	–	–
Total sugar (g/100 mL)	–	–	–	–	1.75	–	–	–	–
TPC (mg GAE/mL)	–	–	–	7.6	0.41 <sup>e</sup>	–	–	–	374.13 <sup>f</sup>
TSS (°Brix)	5.2	–	–	–	5.9	–	–	–	9.67
Viscosity (cP)	52.5	–	–	–	–	–	–	4.68–17.9 <sup>g</sup>	–

Parameter	<i>O. stricta</i> juice	<i>O. ficus-indica</i> wine	<i>Opuntia</i> wine	<i>O. ficus indica</i> alcoholic beverage	<i>Opuntia</i> juice	Colonche	Fermented PPE	<i>O. ficus-indica</i> puree	<i>O. ficus-indica</i> beverage
Volatile acidity (% v/v)	–	–	0.10–0.21	–	–	–	–	–	–
Reference	Castellar et al. (2008)	Tsegay et al. (2018), Tsegay and Lemma (2020)	Gebremedhin et al. (2017)	Karabagias et al. (2020)	Panda et al. (2017)	Rodríguez-Lerma et al. (2011)	Son and Lee (2004)	Di Cagno et al. (2016)	Randazzo et al. (2016)

PPE prickly pear extract

<sup>a</sup>at 535 nm

<sup>b</sup>%

<sup>c</sup>g/L

<sup>d</sup>per 100 mL

<sup>e</sup>µg/mL

<sup>f</sup>mg/L

<sup>g</sup>mPA



1 g/L, and 1.92 g/L, respectively (Table 30.1). Using *L. fermentum* ATCC 9338, a lacto-juice was developed from an *Opuntia* spp. and a 6 mg/100 mL ascorbic acid, 0.32 mg/100 mL lactic acid, 1.7 g/100 mL tartaric acid, and a pH of 4.1 (Panda et al., 2017). TSS, total sugar, and reducing sugar were 5.9°Brix, 1.75 g/100 mL, and 0.20 g/100 mL, respectively (Table 30.1).

For the composition of alcoholic beverages from *Opuntia* spp., Rodríguez-Lerma et al. (2011) reported 8.37% alcohol content in colonche. A range of 7.65–7.78% alcohol content was also reported in *O. ficus-indica* wine and a tartaric acid value of 10.60 g/L (Tsegay et al., 2018; Tsegay & Lemma, 2020). Gebremedhin et al. (2017), in their study of *O. ficus-indica* alcoholic beverage, reported 200–343 mg/L higher alcohol content, pH of between 3.7 and 4.0, and TTA of 0.6–0.74%. Volatile acidity was recorded as 0.10–0.21% and total esters of 20–35 mg/L (Table 30.1). For both alcoholic and non-alcoholic beverages, variations in their composition could be due to differences in sources of the *Opuntia* species, fermentation methods, and other processing conditions.

Several studies have characterized the volatile constituents in *Opuntia* beverages (Table 30.2). Panda et al. (2017) reported the presence of seven ‘risky’ volatile compounds (2-propenenitrile, 2-(acetyloxy), 3,5-dihydroxy-2-methyl, furfuryl alcohol, 4H-pyran-4-one, 2,2-diethyl-3-methyloxazolidine, acetaldehyde, breznkatechin, and furan) in fresh *Opuntia* juice, but fermentation was observed to have either modified them or disintegrated these compounds. Alcohols and esters were the reported volatiles that dominated an alcoholic beverage from *O. ficus indica* (Karabagias et al., 2020). These included 1,3-dioxolane, acetic acid, benzaldehyde, ethanol, butanedioic acid diethyl ester, decanoic acid ethyl ester, dodecanoic acid ethyl ester, ethane, nonanoic acid ethyl ester, octanoic acid ethyl ester, and styrene. In colonche, volatiles reported was an acid (acetic acid), alcohol ((S)-3,4-Dimethylpentanol), esters ( $\beta$ -phenylethyl acetate, E-11-hexadecenoic acid, ethyl ester, ethyl (3Z)-3-hexenoate, ethyl 9-decenoate, ethyl acetate, ethyl decanoate, ethyl heptanoate, ethyl laurate, ethyl octanoate, ethyl succinate, ethyl undecanoate, hexyl acetate, isoamyl acetate, isopentyl decanoate, pentafluoropropionic acid, hexyl ester), phenols (2,4-di-t-butylphenol, phenylethyl alcohol) and diethyl phthalate (Rodríguez-Lerma et al., 2011). Acids, alcohols, aldehydes, aromatic hydrocarbons, esters, ketones, phenol, sulphur compounds, terpenes, and terpenoids, and compounds with diverse functional groups, were detected in an *Opuntia*-based kefir-like beverage (Randazzo et al., 2016).

Apart from specific reported fermented beverages from *Opuntia* spp., and reported composition thereof, fermentation has also been utilized for the enhancement of production of certain compounds from *Opuntia* spp. These include betalains, a naturally occurring pigment in *Opuntia* spp. (Castellar et al., 2003, 2008) and generation of flavonoid derivatives with anti-inflammatory and antioxidant activities (Filannino et al., 2016).

**Table 30.2** Volatiles reported in fermented *Opuntia* beverages

<i>Opuntia</i> beverage	Volatiles	Reference
Alcoholic beverage	1,3-dioxolane, 2,4,5-trimethyl-, 1-butanol, 2-methyl-, 1-butanol, 3-methyl-, 1-propanol, 2-methyl, 2-butanone, 3-hydroxy-, acetic acid, ethyl ester, benzaldehyde, benzeneethanol, ethanol, butanedioic acid, diethyl ester, decanoic acid, ethyl ester, dodecanoic acid, ethyl ester, ethane, 1,1-diethoxy-, hexanoic acid, ethyl ester, nonanoic acid, ethyl ester, octanoic acid, ethyl ester, and styrene	Karabagias et al. (2020)
Colonche	Acetic acid, (S)-3,4-Dimethylpentanol, $\beta$ -phenylethyl acetate, E-11-hexadecenoic acid, ethyl ester, ethyl (3Z)-3-hexenoate, ethyl 9-decenoate, ethyl acetate, ethyl decanoate, ethyl heptanoate, ethyl laurate, ethyl octanoate, ethyl succinate, ethyl undecanoate, hexyl acetate, isoamyl acetate, isopentyl decanoate, pentafluoropropionic acid, hexyl ester, 2,4-di-t-butylphenol, phenylethyl alcohol, diethyl phthalate	Rodríguez-Lerma et al. (2011)
<i>Kefir</i> -like beverage	Acetic acid, decanoic acid, hexanoic acid, octanoic acid, 1-hexanol, 1-octanol, 2-ethylhexanol, 2,3-butanediol, and its isomer, 4-hepten-1-ol, cis-6-nonenol, benzyl alcohol, fenchyl alcohol, glycerol, isobutanol, isoamylalcohol, phenylethylalcohol, 1-octanal, 5-hydroxymethylfurfural, benzaldehyde, decanal, hexanal, nonanal, trans-2-octenal, furfuraldehyde, hydroxyacetone, 1-heptyl acetate, 2(5H)-furanone, 2-phenylethyl hexanoate, ethyl-9-decenoate, ethyldecanoate, ethyl decanoate, ethyl hexadecanoate, ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl nonanoate, ethyl tetradecanoate, hexyl acetate, hexyl hexanoate, isoamylacetate, isoamyl decanoate, isoamyl hexanoate, isoamyl lactate, isoamyl octanoate, methyl salicylate, myristicin, octyl acetate, phenylethylacetate, phenylethyl octanoate, 1-(3-erthylphenyl) ethenone, 6-methyl-5-heptene-2-one, geranylacetone, tymol, 3-(methylthio)propanol, p-cymene, styrene, anethol, citronellol, d-limonene, $\beta$ -farnesene, $\beta$ -phellandrene	Randazzo et al. (2016)

#### 4 Health-Promoting Characteristics of Fermented Beverages from *Opuntia* spp.

*Opuntia* juice's popularity has increased partly due to its recognition as a source of health beneficial constituents (Filannino et al., 2016). Zenteno-Ramirez et al. (2018) reported that *Opuntia* spp. juice can be regarded as a functional food as it contains soluble fibers, sugars, betalains, and ascorbic acid. Available studies in the literature have reported the presence of compounds having antioxidant, antimicrobial, anti-inflammatory, hypoglycemic, and neuro-protective properties in *Opuntia* juices (Galati et al., 2003; Zenteno-Ramirez et al., 2018).

### 4.1 Antioxidant Activity

*Opuntia* spp. juice is a good source of bioactive constituents and is projected as having functional and nutraceutical importance (Cansino et al., 2013). Galati et al. (2003) reported that *Opuntia* spp. juice contains phenolic compounds that are effective against oxidative damage. The juices also vary in color and contain flavonoids, phenolics (Table 30.1), betacyanins, betaxanthins, and antioxidant compounds (e.g., gallic acid, and quercetin) (Castellar et al., 2008; Chavez-Santoscoy et al., 2009; Tsegay et al., 2018; Tsegay & Lemma, 2020). The presence of various carotenoids, betalains (betanin and indicaxanthin), and flavonoids-related compounds (isorhamnetin, kaempferol, and quercetin) have provided antioxidant activity and may protect against human disease (Dehbi et al., 2013). The study of Madrigal-Santillán et al. (2013) has shown that *Opuntia* juice's antioxidant activities can assist in preventing chronic diseases.

### 4.2 Anti-inflammatory Property

The presence of natural flavonoid-rich compounds in *Opuntia* juice is a potential source of anti-inflammatory compounds (Matias et al., 2014; Kotadiya et al., 2018). The concentrated juice was effective against induced-oxidative stress and inflammation in the human intestinal epithelium (Caco-2 cells) (Matias et al., 2014). Alimi et al. (2012) also reported the protective effect of *Opuntia* spp. juice upon ethanol-induced damages in rat erythrocytes, wherein the protective effect was dose-dependent due to the high content of antioxidant compounds (e.g., ascorbic acid, betalains, carotenoids, flavonoids, and phenolics), which reduced the scavenging activity in plasma. The *O. elatior* Mill. juice has shown a protective effect against alterations in the pancreas and liver's biochemical parameters and pathological lesions in diabetic rats (Kotadiya et al., 2018).

### 4.3 Hypoglycemic Effect

The juices are rich in carbohydrates, protein, vitamin C,  $\beta$ -carotene (precursor of vitamin A), and several active compounds that show several pharmacological actions, including cholesterol-lowering, hypoglycemic and antidiabetic effects (Boutakiout et al., 2018). Abdallah (2008) studied the possible curative role of *O. dillenii* fruit juice using induced diabetic rats. The oral administration of juice induced the improvement of lipid profile and significantly reduced blood glucose compared with the non-treated diabetic group (Abdallah, 2008).

**Table 30.3** An overview of *Opuntia* spp. fruit juice associated with health benefits

Active components	Health benefits	Reference
Ascorbic acid, polyphenols, and flavonoids	Antioxidant and antiulcerogenic activity	Galati et al. (2003)
Vitamins and soluble fibres	Hypoglycemic activity	Abdallah (2008)
Total phenolics, flavonoids, betaxanthins, and betacyanins	Prevent oxidative stress and cancer	Chavez-Santoscoy et al. (2009)
Polyphenols, flavonoids, ascorbic acid, carotenoids, and betalains	Reduced the scavenging activity in plasma	Alimi et al. (2012)
Phenolics and flavonoids	Anti-oxidative effect	Dehbi et al. (2013)
Flavonoids	Anti-oxidative and anti-inflammatory	Matias et al. (2014)
–	Anti-inflammatory	Kotadiya et al. (2018)
Tocopherols, flavonoids, and phenolic acids	Antioxidant and antiradical activity	Boutakiout et al. (2018)

#### 4.4 Reduced Risk of Oxidative Stress-Related Diseases

Betalains and flavonoids, compounds naturally present in *Opuntia* juice (Table 30.3), have been associated with reduced risk of oxidative stress-related diseases, such as cardiovascular, cancer, and neurodegenerative diseases (Boutakiout et al., 2018). Chavez-Santoscoy et al. (2009) investigated the juices of prickly pear (*Opuntia* spp.) against four cancer (prostate, colon, mammary, and hepatic) cell lines. The juices were reported to reduce cancer cell growth without affecting normal fibroblast viability.

## 5 Potential for Development of Fermented Beverages from *Opuntia* spp.

Fermented beverages prepared from *Opuntia* spp. are important to the globe. There is a continuous demand for these products, as fermented products are sources of functional foods (Adebo & Medina-Meza, 2020). Thus, there is a need to improve processes to guarantee beverages with better composition and attributes. The use of starter cultures is one way to increase acidification and ensure a product with consistent composition and improved process (Adebo et al., 2018). Further to this is the need to effectively optimize necessary fermentation conditions to ensure an efficient process and desired product quality.

The fourth industrial revolution (4IR) cannot be disregarded, and its utilization in fermented products has not been explored (Adebo, 2020). Machine learning (ML) and artificial intelligence (AI) are 4IR techniques used in other fields, with enormous potential for addressing fermentation challenges. Both ML and AI have

considerable roles to play in fermentation, including predictive product development, improvement in plant productivity and product efficiency, optimization of fermentation processes, and ensuring food safety. Some of these 4IR-related techniques have been demonstrated in other fermentation studies (Chen et al., 2015; Tan et al., 2019; Zhou et al., 2019; Zhu et al., 2019, 2020).

The intricacies of the fermentation process lead to changes in the overall composition of the products. Most fermented food products are characterized by a complex microbiota and an abundant metabolite profile. An adequate understanding of these complex systems, including the microbial diversity, health benefits, nutritional constituents, flavor profile, and metabolic interactions would require a robust system that provides detailed insights into fermented beverages from *Opuntia* spp. Such ‘omics’ techniques that can be adopted for fermented *Opuntia* beverages include metagenomics, which can be used to characterize microbiota and potential gene functions of the microorganisms (Mandhania et al., 2019); metatranscriptomics, for insight into the degree of gene expression and activity; metaproteomics to reveal the nature of microorganism function at the protein level (Yang et al., 2020) and metabolomics which involves the comprehensive analysis of metabolites within a sample (Adebo et al., 2021). Such insights would advance the understanding of fermentation processes and molecular descriptions of resultant food products, vital for improving the existing processes.

## 6 Status and Scope of Commercialization of Beverages from *Opuntia* spp.

The fruit of the *Opuntia* spp. is processed into various products, including juice and fermented beverages. Other products include jam, jelly, canned fruit, and dehydrated fruit products. Countries like Bolivia and Argentina consume the cactus fruits as fresh juice or pulp; neither do they export the fruit nor process it into commercial food products such as jams, jellies, and vinegar (Paiuc, 2017). People of the *Opuntia* spp. fruit-producing countries consume fresh fruit juices, or most of the processed products such as jams and confectionery. Mexico produces bouquets of products from cactus fruits. In Italy, wines and liquors are produced from cactus fruit and sold in a commercial format. However, many other products produced from cactus fruits are still considered as regional. A few manufacturers export processed foods and beverages but not significantly to many countries. The prime reason is the lack of expertise and facilities available with the manufacturers as per the modern requirements. Hence, regulatory and safety aspect is the prime concern. Establishment of an international agency on *Opuntia* spp. such as the International Potato Centre (CIP) could solve the internationalization and popularization of beverages developed from *Opuntia* spp.

## 7 Conclusion

Fermentation is an ancient food processing technology, unique but common to all regions of the world. There is an increasing interest in functional beverages from a scientific, consumer, and commercial perspective. Considering the nutritional compositions and potential health benefits of *Opuntia* spp., formation of cooperative farmer societies would help proper collection of fruits. Further, the local governments could also take the initiative for a common facility center to manufacture beverages from *Opuntia* spp. Commercialization and export of such products would aid in transforming the socioeconomic status of the farmers and local denizens engaged in the cultivation and collection of *Opuntia* spp. fruits.

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# Chapter 31

## *Opuntia* spp. Marmalade



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**Abstract** Prickly pear (*Opuntia* spp.) fruit is highly perishable because of the high moisture and soluble solids content. The utilization of prickly pear fruit to produce preserves like marmalade receives particular attention from food industry. The production of prickly pear marmalade is a complex process and affected by several parameters such as water, pectin, sugar, and acid levels. This chapter aims to review the manufacturing process of prickly pear marmalade.

**Keywords** Marmalade · Jam · Jelly · Pectin · Sugar · Prickly pear

### 1 Introduction

Prickly pear fruit, also called cactus pear fruit, is produced by several species of the *Opuntia* genus belonging to the Cactaceae family. It is native to arid and semi-arid regions of Mexico. The oval-shaped fruit is weighed 95–101 g, with 4.72–7.24 cm in length and 3.29–4.96 cm in diameter (Arba et al., 2002). Depending on the concentrations of betalain and chlorophyll pigments, the fruit colours range from green, orange, red, orange to purple (El Kharrassi et al., 2016). The fruit can be divided into three parts, namely peel (48%), the pulp (45%), and seeds (7%) (Sawaya et al.,

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1983). Glochids cover the thick peel surface, and many hard-coated seeds are found inside the fleshy pulp.

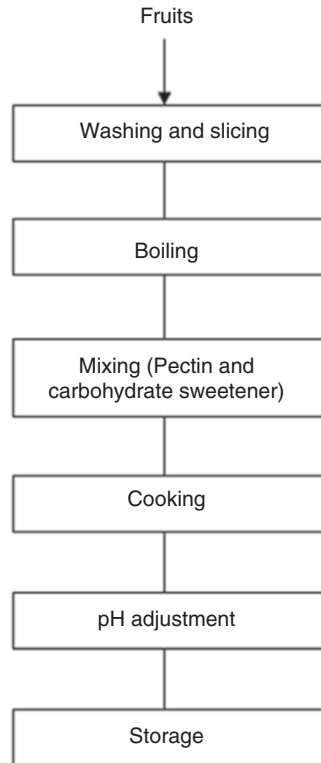
Prickly pear bears fruits throughout the year. Without proper post-harvest storage, the fruit has a very short shelf-life, and it begins to deteriorate after 7–9 days of harvesting (Guerrero-Beltrán & Ochoa-Velasco, 2018). As such, prickly pear fruit is usually processed into value-added products like marmalades, jams, jellies, juices, candies, nectars, vinegar, sweeteners, dehydrated fruit slices, alcohol, and wines (Guerrero-Beltrán & Ochoa-Velasco, 2018). Among these products, the production of marmalades using prickly pear fruit is receiving particular attention. Marmalade is produced from whole fruit or fruit pieces and contains fine shreds of peel (Codex Alimentarius Commission, 2009). It is considered a concentrated form of fresh fruit, albeit with higher sweetness and acid content than their fresh counterparts. It is also a useful alternative for other high-calorie spreads like butter, and chocolate (Egbekun et al., 1998). This chapter aims at reviewing the manufacturing process of prickly pear marmalade.

## 2 Marmalade, Jam, and Jelly

Marmalade, jam, and jelly, collectively known as preserves, are produced from various fruit ingredients. Globally, Europe is the largest market for these preserves, representing about 50% of the total world imports (Centre for the Promotion of Imports from Developing Countries-CBI, 2018). These products are sometimes misidentified by consumers due to the shared characteristics like gelled consistency and high soluble solids content. According to the Codex Alimentarius Commission (2009), marmalade is produced from whole fruit or fruit pieces and contains fine shreds of peel, whereas jam is produced from the fruit pulp. Meanwhile, jelly is a crystal-clear jam produced from fruit juice. Water and carbohydrate sweeteners such as sugar, honey, or fructose syrup are usually added to these preserves during the manufacturing process.

Generally, marmalades can be classified into citrus or non-citrus, depending on the source of ingredients used. Citrus marmalade is produced using single or mixed citrus fruits such as oranges, lemons, grapefruits, and mandarins. The sales volume of citrus marmalades was about 26,000 tons in 2018 (Wunsch, 2019). Production of citrus marmalade uses either whole fruit, which might have had all or part of the peel removed, or fruit pieces such as peel, pulp, and juice (Codex Alimentarius Commission, 2009). In contrast, non-citrus marmalade is produced using non-citrus fruits such as prickly pears and apples. The non-citrus fruits are cooked either in whole or in pieces before further processing into marmalades (Codex Alimentarius Commission, 2009).

**Fig. 31.1** Flowchart of marmalade production



### 3 Marmalade Production

The general steps in marmalade production have been described by Kamiloglu et al. (2015). The fruits are washed, cut into thin slices, and boil with water for 20–30 min. Pectin and carbohydrate sweeteners are then added to the mixture before continuing cooking at mild temperatures. Fruit acid, such as citric acid, malic acid, or tartaric acid, is then added to lower the mixture's pH. After cooling to room temperature, the mixture is store in sterilized bottles. Figure 31.1 shows the flowchart for marmalade production.

### 4 Prickly Pear Fruit Ingredients in Contributing Marmalade Production

The production of an excellent prickly pear marmalade is a complex process. It is influenced by the correct combination of water, pectin, sugar, and acid content. Detail discussion of each aspect is as follows.

## 4.1 Moisture Content

Moisture content is a measurement of the total amount of water in food samples. The water requirement during the manufacturing of preserves depends on the natural properties of fruits. For instance, the addition of water is required for firm fruits (e.g., prickly pear and apple), but not for juicy fruits (e.g., berries) (Smith, 2005). More than 90%, in dry weight basis, of the peel and the pulp is contributed by moisture content (Table 31.1). The addition of water to firm fruits during the cooking process of marmalade is to promote fruits softening. High moisture content in the peel and the pulp of prickly pear fruit suggests that a low amount of water is added during the cooking process.

**Table 31.1** Nutritional composition of prickly pear fruit (dry weight basis)

Component	Prickly pear		
	Peel	Pulp	Seed
Moisture content (%) <sup>a</sup>	90.33	94.40	18.05
<b>Fibers (%)<sup>b</sup></b>	<b>40.80</b>	<b>20.50</b>	<b>54.20</b>
Pectin	3.14	14.41	3.62
Lignin	0.02	Trace	0.10
Cellulose	29.13	2.91	45.09
Hemicellulose	8.49	3.18	5.39
Total soluble solids (°Brix) <sup>c</sup>	8.03–15.40	10.70–15.70	NR
<b>Carbohydrates (%)<sup>a, b</sup></b>	<b>18.54–26.25</b>	<b>53.19–64.82</b>	<b>0</b>
Glucose	14.00–21.00	29.00–35.00	ND
Fructose	2.25–2.89	24.00–29.60	ND
Saccharose	2.29–2.36	0.19–0.22	ND
Mannose	ND	ND	ND
Raffinose	ND	ND	ND
Stachyose	ND	ND	ND
Galactose	ND	ND	ND
Xylose	ND	ND	ND
pH <sup>c</sup>	4.83–5.59	5.41–6.15	NR
Titrateable acidity (% citric acid) <sup>c</sup>	0.61–3.40	0.23–1.63	NR

ND not detected, NR not reported

<sup>a</sup>Salim et al. (2009)

<sup>b</sup>Kossori et al. (1998)

<sup>c</sup>Andreu et al. (2018)

## 4.2 *Pectin Content*

Pectin is a type of structural fiber found in the intracellular layer and primary cell wall of fruits. It is charged, hydrophilic, and able to form a gel once the correct concentrations of sugar and acid are reached. The pectin's gel-forming characteristic is due to its capability to form a three-dimensional network through cross-linking between the chains (Berk, 2016). This characteristic can indirectly affect the final texture of marmalade. The recommended pectin concentration is 0.5–1.5% by weight of marmalade (O'Beirne, 2003). Utilizing fruits with adequate amounts of natural pectin can form a gel in the marmalade, even though without the addition of synthetic or commercial pectin. Thus, it is crucial to understand the pectin content of prickly pear fruit.

Four fiber components, namely, pectin, lignin, cellulose, and hemicellulose, were isolated in the prickly pear fruit (Table 31.1). The pulp's main component was pectin, accounting for more than 70% of the total fiber content. According to Goycoolea and Cárdenas (2003), the pectin content of prickly pear was comparable to pectin's commercial sources such as citrus peel and apple pomace.

## 4.3 *Total Soluble Solids and Carbohydrate Composition*

Total soluble solids are usually used to measure the sugar content of food samples. The recommended minimum total soluble solids content for marmalade is 60°Brix, equivalent to the sugar content of 60% (O'Beirne, 2003). The correct quantity of sugar content is essential for preserving mouthfeel and proper gel formation of the marmalade. Marmalade with low total soluble solids (<60°Brix) will have a runny consistency, and food-borne pathogens might grow. Hence, it is essential to understand the total soluble solids nature of prickly pear fruit to determine the amount of carbohydrate sweeteners added during marmalade production. An early study of Sawaya et al. (1983) reported that the total soluble solids of prickly pear pulp were comparable to the common fruits (e.g., cherry, plum, apple, apricot, strawberry, and raspberry) used in making preserves. The average total soluble solids of the pulp (13.07°Brix) of six different prickly pear cultivars were greater than the peel (11.81°Brix) (Andreu et al., 2018).

The sugar content in the marmalade comes from the fruits and the added carbohydrate sweeteners. Ethanol-soluble carbohydrates are the most abundant compounds in the peel and the pulp of prickly pear fruits (Yahia & Saenz, 2017). This attribute is advantageous, especially in marmalade manufacturing, as fewer carbohydrate sweeteners will be required. By using high-performance liquid chromatography, glucose, fructose, and saccharose were detected in the peel and the pulp of prickly pear (Table 31.1). The pulp contained higher amounts of glucose and fructose than the peel and the seed. The presence of glucose and fructose suggests prickly pear fruits' suitability to be utilized as natural carbohydrate sources of

sweetness for food preparations. Meanwhile, carbohydrates such as galactose, stachyose, and raffinose, known for causing intestinal discomfort (Kossori et al., 1998), were absent in the prickly pear fruits.

#### **4.4 Titratable Acidity and pH**

Titrateable acidity and pH are interrelated concepts in food analysis that deal with acidity. Titrateable acidity measures the total acid concentration in food samples, whereas pH measures acids' strength in food samples. High acidity is necessary to prevent the growth of pathogenic microorganisms, promote storage stability, and assure a satisfactory setting of marmalade. A previous study reported that low pH could prevent sugar crystallization in the marmalade during storage (Saenz, 2000). However, according to Berk (2016), high acidity (pH < 2.8) in the fruit preserves is not favorable as it promotes shrinkage of the pectin network and causes syneresis, a condition characterized by oozing of liquid from a gel structure. Therefore, it is essential to maintain the marmalade at optimum pH levels between 2.8 and 3.3 (Bourne, 1999).

Prickly pear peel and pulp are classified as weak acids (pH > 4) and have very low titrateable acidity contents (Table 31.1). Non-citrus fruits are generally low in acid content, and the addition of fruit acid is necessary to bring pH into the correct range for gel formation and flavour purposes during the manufacturing of prickly pear marmalade. As citric and malic acids are the primary organic acids detected in the peel and the pulp of prickly pear fruits (García et al., 2020). It is recommended to use citric acid or malic acid to adjust the pH of marmalade.

### **5 Prickly Pear Marmalade Production**

The general steps for marmalade production are summarized in Fig. 31.1. The nutritional composition varies in different types of fruits; thus, the recipe for prickly pear marmalade production may be different. As the thick peel constitutes the largest portion of the prickly pear fruit, commercial prickly pear marmalade is produced using either the peel alone or the combination of the peel and the pulp (Cerezal et al., 2007). The hard-coated seeds are usually used to produce edible oil (Ennouri et al., 2006; Tan et al., 2020).

The ripe, free of spines and glochids prickly pear fruits, is initially washed under water to remove the surface's dirt. The fruits are then unpeeled and cut into small shreds, whereas the pulp is pressed into juice using a juice extractor. A strainer is used to separate the seeds from the juice. The small shreds peel and/or the juice is then boiled for 20–30 min. A pectin test is usually carrying out to determine the amount of pectin that has been extracted from the fruit. The process continues by adding pectin power and carbohydrate sweetener into the mixture. The mixture is

cooking at mild temperatures for another 10–20 min. Afterward, the pH of the mixture is adjusted to the range of 2.8–3.3 using commercial fruit acid. The mixture is stored in sterilized bottles after attaining room temperature. On the other hand, prickly pear fruits also combine with other fruits such as orange and pineapple to produce mixed fruit marmalades. Detail recipes can refer to the book published by Willoughby and Willoughby (1998).

## 6 Conclusion

Marmalade is a gel consistency fruit preserves with fine peel shreds within. The production of an excellent prickly pear marmalade is influenced by the right balance of water, pectin, sugar, and acid ingredients. Up to date, there is no study on optimizing these ingredients and processing parameters for the production of prickly pear marmalade. Further study is also necessary to validate the influences of various storage conditions on the physicochemical properties of prickly pear marmalade.

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## Chapter 32

# *Opuntia* Pear Peel as a Source of Functional Ingredients and Their Utilization in Meat Products



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**Abstract** *Opuntia ficus-indica* is cactus extensively cultured in Mexico. Besides the amply consumed leaf, called nopales, the pear as the fruit is consumed during the season from July to September, producing approximately 35,200 tons. Since the thick and spiny *Opuntia ficus* pear peel is not consumed, it represents a co-product that can be exploited as a source of fiber and other bioactive compounds, already evaluated as functional ingredients to enhance the quality of meat products. *Opuntia* pear peel total dietary fiber content is above 60%, with high insoluble fiber content and high prebiotic potential. *In vivo*, the prebiotic effect was demonstrated feeding rats that grow similar inulin diet as control. *Opuntia* pear peel was employed as a fiber source in cooked sausages, improving yield with no detrimental effect on texture, and allowing the proliferation of inoculated thermotolerant lactic acid bacteria. In this view, *Opuntia* pear peel is considered a functional ingredient that enhances the physicochemical and nutritional characteristics of meat products.

**Keywords** *Opuntia ficus-indica* · Fiber · By-products · Prebiotic · Fatty acids · Phenolics

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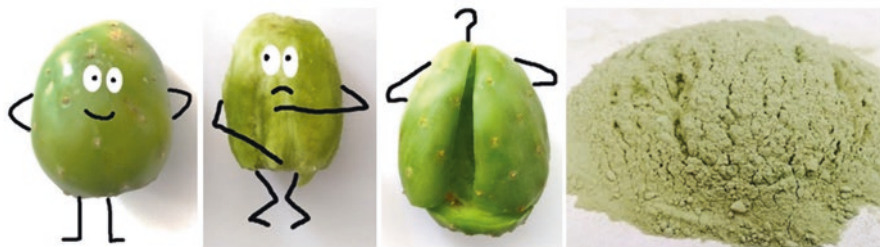
M. F. Ramadan et al. (eds.), *Opuntia spp.: Chemistry, Bioactivity and Industrial Applications*, [https://doi.org/10.1007/978-3-030-78444-7\\_32](https://doi.org/10.1007/978-3-030-78444-7_32)

## 1 Introduction

There is an interest in the generation of many agricultural byproducts that could be used as a source of food ingredients. In recent years, there has been a global trend to natural plant food components, mainly dietary fiber and phytochemicals, both found in *Opuntia ficus* pear. Byproducts are a natural source of the functional ingredients, like antioxidants and bioactive compounds, with a crescent interest not only by the nutritional quality but also by its cost and additional benefits, since the use of byproducts as useful ingredients could make them an added-value products (Bensadón et al., 2010).

The physicochemical properties of peel fiber can be used in the food industry to improve shelf-life, sensory attributes, staling, and viscosity, serving as water retention agents and oxidative stability enhancers. Since peels are cheap and relatively available, commercialization potential is plausible (Aruwa et al., 2018). The process of *Opuntia* pear results in the increase of several byproducts, representing around 30% of the fruit weight (peel mainly), containing several valuable bioactive compounds and pectin. Low-methoxyl pectin had been extracted from peels with the composition of the following sugar (in dry weight): galacturonic acid (654 mg/g), galactose (195 mg/g), rhamnose (21.6 mg/g), arabinose (1.2 mg/g), and glucose (1.2 mg/g) (Villacís-Chiriboga et al., 2020).

The consumption of “nopal” in Mexico is ancestral, being one of the centers of origin of this plant. Tenochtitlan was founded in a swamp where Aztecs saw an eagle eating a snake stand on a nopal cactus. Nopal plants can adapt to moisture insufficiency and semi desertic weather conditions, and the edible cactus stem is widely consumed almost all the year. Nopales possess some nutraceutical characteristics besides a higher vitamin C content, minerals, and soluble and insoluble fiber. The edible stem’s quality characteristics are regulated by Mexican norm NMX-FF-068-SCFI-2006 and the international norm Codex Stan 185-1993 (Maki-Díaz et al., 2015). The cactus pear season runs from April to September, and in 2010, Mexico’s central region consumed 47% of the total production (398,361 kg), with an average annual consumption of 4.28 kg per capita (Diaz-Vela et al., 2013). The production potential of this crop in the central area of Mexico, an area dedicated to cactus pear production, generated a significant economic benefit in the purchase of negotiable inputs and domestic factors where manual labor is the most important. Thus, the fruit is a very demanding job due to the nature of the product obtaining process, and according to the opinion of the producers, so the results show that the production units on average were profitable (Ramírez Abarca et al., 2015). In Mexico, 20,000 producers harvest, on average, 352,000 tons in 48,000 h available for the prickly pear cactus, being the center of the country (Estado de Mexico, Puebla, and Hidalgo) main production area close to the megalopolis (Granillo Macías et al., 2019). The fruit is consumed fresh (9–15 days after harvest), and peels are discarded (Fig. 32.1). Peels were also employed in the development of marmalade as a pectin source. The peel pH is between 5.4 and 5.8 (Cerezal & Duarte, 2005).



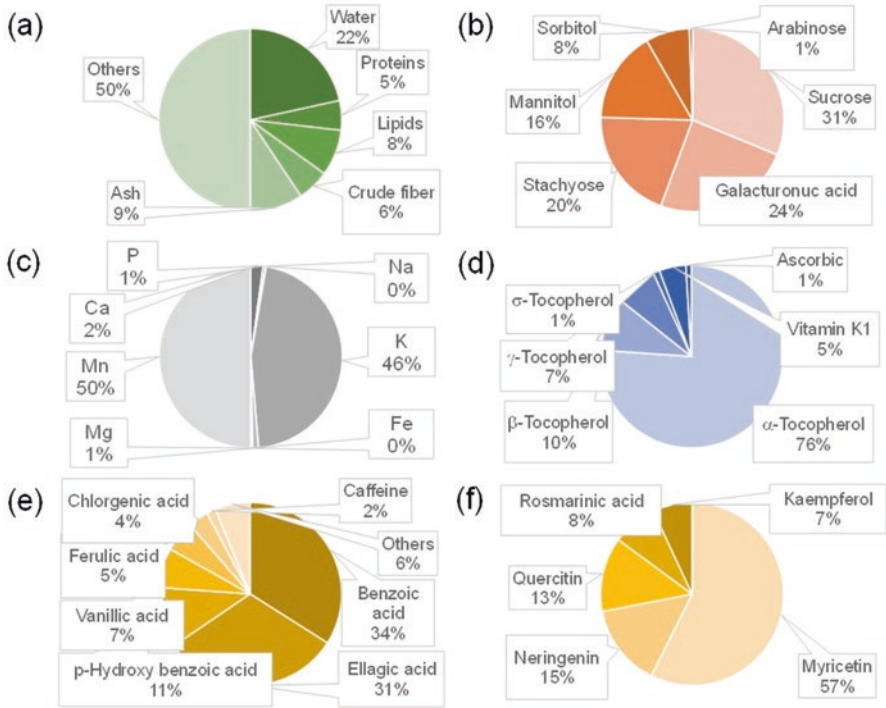
**Fig. 32.1** Cactus prickly pear or cactus pear fruit, unskinned, peel, and the flour obtained after dry, mill and sieve

## 2 *Opuntia* Peel Composition

Peel represents 40% of the pear weight, and lipid content is 3.68% dry weight, where neutral lipids are predominantly, besides a considerable content of linoleic, oleic, and palmitic acids. Soluble dietary fiber fraction in peel represents, on average, around 7% dry weight, constituted mainly by mucilage, with a high content of galacturonic acid. The dietary fiber in peel is associated with minerals in plant cells, wherein minerals content in peels is higher than in pulp, being rich in potassium, phosphorus, magnesium, and calcium. Similarly, all parts of the fruit are rich in phenolics, flavonoids, and phenolic acids mainly. The isorhamnetin-3-*O*-rutinoside is the main flavonol glycosides present in peels. Other antioxidant compounds also present in higher proportion in the peel than in the rest of the fruit including carotenoids, betacyanins, phenolics, and vitamin C (Barba et al., 2020). The content of bioactive compounds like isorhamnetin glycosides is greater in peels than in pulp (García-Cayuela et al., 2019). There was no glucose in *Opuntia* ficus peel (Salim et al., 2009). In this view, the peel is rich in many nutrients and bioactive compounds. On a dry basis, crude fiber is one of the main components. Sugars' composition includes oligosaccharides and other sugars. The relative amount of main phenolics also indicate that peels are a good source of antioxidants. Also, a relatively high proportion of vitamin K and vitamins E are present in peel (Fig. 32.2).

### 2.1 *Lipids and Fatty Acids*

In peel extracts, 14 acids have been identified, whereas the major fatty acids are linoleic, followed by oleic and palmitic acids (*ca.* 75%). There was a relatively high level of polyunsaturated fatty acids, trienes in particular, with  $\gamma$ -linoleic fatty acid was measured in a higher level than  $\alpha$ -linoleic acid. Low content of long-chain fatty acids (behenic C22:0, lignoceric C24:0, docosadienoic C22:2, cerotic C26:0, and



**Fig. 3.2.2** Chemical composition (a: El-Salid et al., 2011) and main components relative percent of compounds as sugars (b: El-Salid et al., 2011), minerals (c: El-Beltagi et al., 2019), vitamins (d: Slimen et al., 2016), phenolic compounds (e: El-Beltagi et al., 2019), and flavonoids (f: El-Mostafa et al., 2014)

nervonic C24:1) was also detected. Linoleic acid was the major component in neutral fatty acids, whereas palmitic acid was the major component of the polar fraction (Ramadan & Mörsel, 2003).

## 2.2 Pigments

The *Cactaceae* family contains the betalains as natural pigments. These pigments' structure is derived from the betalamic acid and depending on the united components to this structure, the yellow betaxanthins and the red-violet betacyanins formed (Díaz-Sánchez et al., 2006). Core pigmentation occurs first and well before fruit maturity and peels pigmentation. Peel pigmentation is fully developed at maturity, presumably related to maximum soluble solids (Felker et al., 2008).

## 2.3 Functional Ingredients

Functional ingredients are a diverse group of compounds that are intended to produce a positive effect on the consumer's health.

### 2.3.1 Antioxidants (Phenolic Compounds)

Phenolic compounds are the main class of secondary metabolites in plants and divided into phenolic acids and polyphenols. The polyphenols have a strong relationship with the potential antioxidant of fruits and vegetables. Cardador-Martínez et al. (2011) studied the antioxidant capacity and polyphenols in seeds and peel of cactus pear. They reported that the polyphenols were higher in the peel than in the seeds (362 mg gallic acid/100 g dry peel). Generally, fruits with light-green or yellow-brown peel have higher antiradical activity and Trolox equivalent antioxidant capacity (TEAC) values than those with the red-purple peel.

Peels from *Opuntia ficus indica* var. *sanguigna*-OS, *Opuntia ficus indica* var. *giabella*-OG, and *Opuntia engelmannii*-OE contain 12 different phenolic compounds including two phenolic acids (piscidic acid, and eucomic acid), and ten flavonoids, as isorhamnetin, quercetin and kaempferol derivatives, and isorhamnetin-*O*-(deoxyhexosyl-hexoside) as the major compound found. These byproduct active compounds possess intrinsic antioxidant and antimicrobial activity (Melgar et al., 2017). Since more polyphenols and phenolic acids are present in the peel, this biologically active polyphenolic and betacyanins mixture have a high antioxidant activity (Yeddes et al., 2013).

### 2.3.2 Total Dietary Fiber

Dietary fiber is that part of plant material in the diet, which is resistant to enzymatic digestion, including cellulose, noncellulosic polysaccharides such as hemicellulose, pectic substances, gums, mucilages, and non-carbohydrate component lignin (Dhingra et al., 2012). There are two types of fiber: the soluble fiber solubilized in water and the insoluble fiber that cannot be solubilized in water. The sum of both is the total dietary fiber. Dietary fiber has health benefits. The soluble fiber is related to lower fat absorption, lower cholesterol, stabilizing blood sugar levels. The insoluble fiber can also be beneficial for various digestive conditions associated with sluggish or irregular bowel movements. Peel fruits are a good source of dietary fiber. Table 32.1 shows the content of dietetic fiber in the different peel of fruits. The drying method affects the fiber composition of *Opuntia* peel, with a higher content in insoluble dietary fiber (40.7%), soluble dietary fiber (13.3%), and total dietary

**Table 32.1** Content of dietetic fiber in different peel of fruits

Peel fruit	Total dietary fiber	Soluble dietary fiber	Insoluble dietary fiber	Reference
Rambután	52.97	10.22	42.74	Wanlapa et al. (2015)
Kaeo mango	67.84	29.54	38.30	Wanlapa et al. (2015)
Raspuri mango	54.9	17.2	37.7	Ajila and Prasada Rao (2013)
Apple peel	39.7	–	–	Henriquez et al. (2010)
Pineapple peel	64.15	33.67	30.48	Diaz-Vela et al. (2013)
<i>Opuntia ficus</i> peel	62.54	21.88	40.66	Diaz-Vela et al. (2013)

fiber (44%) in hot air-dried as compared to freeze-dry (34, 6 and 40) (García-Amézquita et al., 2018).

### 2.3.3 Prebiotics

A prebiotic is “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health” (Roberfroid, 2007). Among prebiotics, inulin is a soluble (98%) and fermentable fiber named fructan that reaches the large intestine practically intact, is then hydrolyzed in the upper section of the intestine and fermented by bacteria (de Souza Oliveira et al., 2011). Agroindustrial co-products contain a significant amount of insoluble fiber. The lactic acid bacteria have shown a diauxic growth in the presence of insoluble fiber. The adaptation time that the microorganism needs to activate the enzymes necessary to metabolize the second substrate. Diaz-Vela et al. (2013) studied the growth effect of two probiotic lactic acid bacteria (*Pediococcus pentosaceus* and *Aerococcus viridans*) using *Opuntia* pear peel as a carbon source. The results showed that the generation time for *Pediococcus pentosaceus* was 0.66 h<sup>-1</sup> using glucose and 0.71 h<sup>-1</sup> using *Opuntia* pear peel, and for *Aerococcus viridans*, it was 0.9 h<sup>-1</sup> using glucose and 1.05 h<sup>-1</sup> using *Opuntia* pear peel. The slower growth is due to the amount and type of carbohydrates present that makes them show diauxic growth. The results demonstrated the potential of *Opuntia* pear peel as a prebiotic *in vitro*. In the same manner, lactic acid bacteria and bifidobacteria counts were higher in *Opuntia* pear peel flour due to higher insoluble fiber (Pérez-Chabela et al., 2015).

The treatment with high hydrostatic pressure changes the composition of the sugars of *Opuntia ficus* pear peel. Tejada-Ortigoza et al. (2019) reported an important reduction of arabinose after the treatment due to the transformation of water-insoluble xyloglucans into water-soluble xyloglucans. However, in the *in vitro* fermentation, no significant change in short-chain fatty acids production between treated or untreated samples was observed.

## 2.4 Health Benefits of *Opuntia ficus Peel*

The anti-toxicity effect was related to blood serum, and serum creatinine decreased due to the higher content of ascorbic acid in the peel. Also a decrease in liver enzymes (AST and ALT) and LDL-cholesterol was observed in rats fed with *Opuntia* peel due to fiber content (El-Salid et al., 2011).

### 2.4.1 Hypocholesterolemic Activities

Regarding the *in vitro* studies of peel extract in diet, supplementation of peel extract increased excretion of cholesterol and decreased liver cholesterol levels (Aruwa et al., 2018). In hamster fed with a hypercholesterolemic diet with *Opuntia* pear peel extract compared with a hypercholesterolemic diet with phytosterols, the results indicated no difference in average cholesterol excretion in male hamsters. However, *Opuntia* peel extracts' effect was much higher in female hamsters, increasing the excretion of neutral sterols as cholesterol and coprostanol with this diet (Milán-Noris et al., 2016). Pérez-Chabela et al. (2015) evaluated the effect of *Opuntia* pear flour and apple marc flour on Wistar rats growth during 90 days, employing inulin as control, reporting that apple marc and *Opuntia* pear peel flour had the same hypocholesterolemic effect as inulin, although with a lower-body gain.

### 2.4.2 Antimicrobial Activity

Hydroalcoholic extracts of *Opuntia* peel demonstrated an excellent antimicrobial effect against Gram-positive bacteria, and specifically against *P. aeruginosa*, as the most inhibited Gram-negative bacteria, with a minimum inhibitory concentration in the 2.50–18.7 mg/mL range (Aruwa et al., 2019).

### 2.4.3 Anticarcinogenic Activity

Peel extracts with higher anticancer activity were reported for chloroform (98%) and ethanol (83%) extracts, exhibiting a dose-dependent anticancer activity in Ehrlich Ascites Carcinoma Cells (EACC) (Abou-Ellella & Ali, 2019). In hydroalcoholic extracts, the presence of isorhamnetin derivatives exhibited a cytotoxic effect on human colon cancer cells (HCT-116) (Serra et al., 2013).

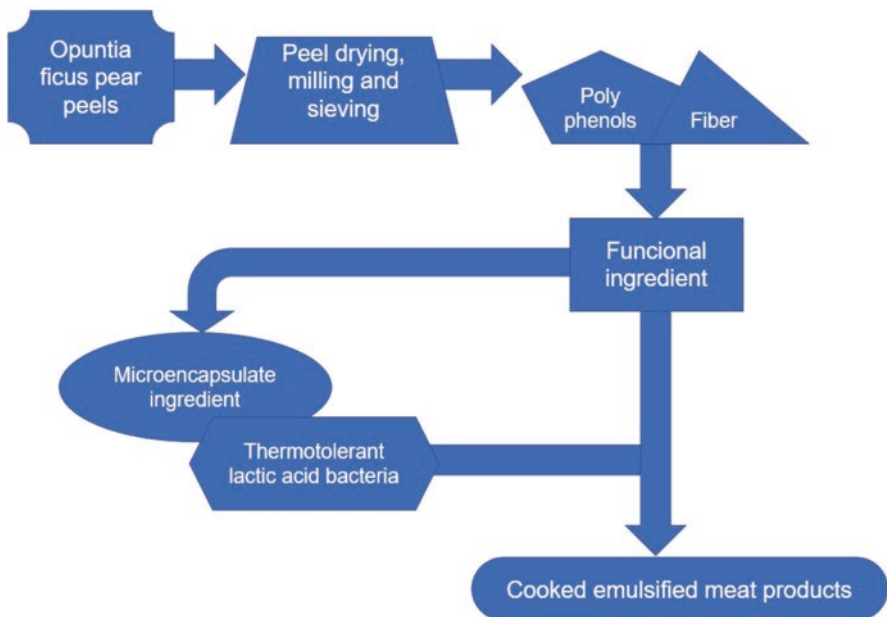


### 3 Functional Meat Products

The population highly consumes cooked meat products due to their low cost and adequate protein intake, and ease of preparation. Thus, enriching them with functional ingredients has been studied.

#### 3.1 *Opuntia ficus* in Meat Products as Functional Ingredients

The use of *Opuntia ficus* as a source of functional ingredients (fiber and prebiotic) is described in Fig. 32.3. *Opuntia ficus* peels are dried, milled, and sieved to obtain a fine powder, named flour (Chávez-Zepeda et al., 2009). The flour is an important source of fiber and fermentable sugars with a good *in vitro* prebiotic score and polyphenols with antioxidant capacity (Díaz-Vela et al., 2013; Parra-Matadamas et al., 2015). Jin et al. (2011) carried out a study incorporating *Opuntia ficus* powder in sausages. Four formulations were made using 0, 1, 5, and 10% of the powder. The results showed that the incorporation of 0.5% *Opuntia ficus* powder increased water holding capacity values during 15 days of storage, with higher luminosity values but no effect on redness during the same storage period, being as hard as the control sample. In other studies, the incorporation of 0.3% of *Opuntia ficus-indica* var. *saboten* powder did not affect the texture properties of cooked sausages but



**Fig. 32.3** Application of *Opuntia ficus* pear peel flour as a functional ingredient in cooked and emulsified meat products

provided the highest lightness (Yoon et al., 2014). Ocampo-Olalde et al. (2015) studied the effect of the inclusion of pear peel flour on the physicochemical parameters in low fat and sodium sausages (2.5% and 5%), with no differences in the total moisture and expressible moisture, although the color became lighter with the inclusion of pear peel flour. Also, Jeon and Han (2019) utilized another *Opuntia* variety (*O. humifusa* f. *jeollaensis*) fruit powder as a source of dietary fiber and color pigmentation in sausage production to improve quality characteristics, including cooking loss and emulsion stability, affecting moisture and cooking loss, water retention capacity, and emulsified sausage texture.

### 3.2 *Microencapsulation*

Because the *Opuntia* pear's skin represents more than 52% of fruit waste, and they have a large number of functional ingredients, and alternative for their use is microencapsulation. Among all microencapsulation techniques, spray drying has become a successful industrial drying process that has evolved as the main technique for the microencapsulation of food ingredients due to its low processing cost, rapid water evaporation, and easy scaling up (Gómez et al., 2018). Toledo-Madrid et al. (2019) studied the effect of storage on the stability of bioactive compounds in spray-dried prickly pear peel stored for 90 days at 22–25 °C. The use of maltodextrins or Arabic gum as encapsulating agents can be used to obtain a high concentration of bioactive compounds giving adequate protection during drying and storage processes, extending the shelf life of bioactive compounds with simple but effective process technology. Although *Opuntia ficus* pear juice had been encapsulated with soluble fiber [(1-3)(1-4)  $\beta$ -D-glucan] from barley as a carrier for spray drying at 22.5%, the use of fruit peel flour could be a better and cheaper source of co-encapsulant (Ruiz-Gutiérrez et al., 2014). Ethanol extracts of pulp were also spray-dry microencapsulated to incorporate phenolic compounds into functional foods (Saénz et al., 2009).

### 3.3 *Synbiotic Meat Product Using Opuntia ficus Peel*

Serrano-Casas et al. (2017) studied the viability *in vitro* of symbiotic (probiotic lactic acid bacteria more cactus pear peel flour as prebiotic) co-encapsulated in alginate. Lactic acid bacteria viability was improved, enhancing the resistance to acidic conditions. Larger microcapsule size (close to 100  $\mu$ m) was related to better viability and longer times resisting acid conditions. Also, symbiotic co-gelification of alginate gel matrix with prebiotic compounds to protect probiotic can be employed to ensure the delivery of probiotic strains in the colon throughout the gastrointestinal tract (Barragán-Martínez et al., 2020). Similarly, Díaz-Vela et al. (2015) studied the physicochemical and structural characteristics of sausages with pear peel flour and *P. pentosaceus*, a probiotic lactic acid bacterium. The results showed that low

rancidity is detected due to the composition of cactus pear peel flour. The lactic acid bacteria increased significantly after 9 days of storage. The samples were significantly harder than the control. This is due to the *P. pentosaceus* produces a remarkable number of exopolysaccharides.

*Opuntia ficus* pear peel flour can be incorporated directly in meat batter formulation and thermotolerant lactic acid bacteria to obtain a symbiotic functional food. Barragán-Martínez et al. (2020) studied the addition of the alginate-pectin microcapsules containing cactus pear peel flour in cooked meat products thermotolerant lactic acid bacteria. The results showed that the microcapsules increased total moisture (from 66% to 75%), inoculated samples presented higher lactic acid bacteria populations since, in addition to the thermotolerant capacity of the bacteria, encapsulation added a protective barrier for the bacteria to survive fewer coliforms in inoculated samples after 15 days of storage. The oxidative rancidity decreased during the storage. These results indicate that the pear peel *Opuntia ficus*'s ionotropic gelation is a good form employed in the probiotic action of cooked sausages.

### 3.4 Sensory Properties

The sensory analysis of new products is essential for subsequent acceptance by consumers and the functional food market. Jin et al. (2011) performed a sensory evaluation of sausages with *Opuntia ficus* flour, where samples containing this ingredient received higher evaluation scores than the control. The scores for color, flavor, tenderness, and juiciness were the highest for the 5% powder group, which may be due to the increased water-binding capacity that, in turn, increased the juiciness. Neophobia is a term that refers to a fear of new. Functional meat products represent a type of food neophobia. Díaz-Vela et al. (2017) studied the neophobia in sausages formulated with cactus pear peel flour. Female consumers aged between 40 and 50 years showed higher interest in the consumption of healthy foods. However, the percentage of neophobia was 50%, which indicates that consumers are not very accessible to new meat products.

## 4 Conclusion

*Opuntia* pear peel can be used as a functional ingredient in meat products due to its high amount of phenolics and fiber, which can act as a prebiotic. Any country with an extensive production of *Opuntia* as México can reduce the generation of agro-industrial waste. Employing the peel as an added-value product to enhance meat products' nutritional properties as a fiber source also has prebiotic potential, beside the antioxidant potential.

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## Chapter 33

# *Opuntia* spp. Seed Oil



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**Abstract** The *Opuntia*, commonly known as cactus pear or prickly pear, belongs to the *Cactaceae* family and is widely distributed either as indigenous, alien, wild, or domesticated species in various countries across the world. Seeds are usually removed as waste products from the fruit pulp and can constitute important new oil source. The *Opuntia* seed oil, commonly called prickly pear seed oil, has been extracted using maceration-percolation, Soxhlet, cold pressing, supercritical carbon dioxide, and ultrasound extraction, for which yields of 1–20% have been reported. *Opuntia ficus-indica* is the most common *Opuntia* species for which the physico-chemical characteristics, the composition of fatty acids, sterols, and tocopherols have been reported. The main fatty acids of prickly pear seed oil are palmitic, stearic, oleic, and linoleic acids. Environmental conditions and maturation stages of prickly pear have effects on the properties of the oil. High levels of sterols are present, with  $\beta$ -sitosterol as the dominant sterol. The dominant tocopherol is  $\gamma$ -tocopherol. The oil exhibited a high *in vitro* antioxidant potential, and with its reported phenolic content, it has various health and cosmetics applications.

**Keywords** Prickly pear · Cactus fruit · Linoleic acid · Antioxidants · Tocopherols · Phenolics · Health · Cosmetics

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## Abbreviations

2-MeO	2-methyloxolane
MP	Maceration-percolation
scCO <sub>2</sub>	Supercritical carbon dioxide
USM	Ultrasound-assisted maceration

## 1 Genus *Opuntia* as a Source of Seed Oil

The genus *Opuntia* generally called the cactus pear, or prickly pear belongs to Cactaceae plant family that includes approximately 130 genera with about 1500 species (Guedes Paiva et al., 2016). They are native to Mexico and widespread throughout Central and South America, Australia, Africa, including the Mediterranean area (Ammar et al., 2014; Shedbalkar et al., 2010). Mexico has the highest genetic diversity of *Opuntia* spp. with 150–180 species, of which *O. hyptiacantha* F.A.C. Weber, *O. leucotricha* DC., *O. megacantha* Salm-Dyck, and *O. streptacantha* Lem. are the most common species (Martínez-Tagüeña & Trujillo, 2020). *Opuntia* spp. are cultivated over a broad range in latitude, and it is growing in areas ranging from the sea level to a height of 5100 m (Guedes Paiva et al., 2016). *Opuntia* tends to favor dry, hot areas inhabited with perennial shrubs, trees, and creeping plants (Shedbalkar et al., 2010). The several varieties of the commonly researched *O. ficus-indica* (L.) Mill. are distinguished by characteristics such as having spiny or spineless cladodes, a cladode shape, branching, its fruit and pulp color, epicuticular wax morphology with sweet pulp and limited acidity, and variable weight of the fruit ranging from 43 to 220 g (Ayadi et al., 2009; Medina-Torres et al., 2013; Guedes Paiva et al., 2016; Cota-Sanchez, 2016). This cactus plant's fruit is elongated and oval, with the pulp containing hard seeds (Piga, 2004). The seeds that are a waste product after pulp extraction and conversion can extract edible oil with unique properties. *Opuntia* seed oil has a high content of unsaturated fatty acids (especially polyunsaturated), dominated by linoleic acid and tocopherols, particularly  $\gamma$ -tocopherol (Stintzing et al., 2001; Simopoulos, 2002; Chahdoura et al., 2015). The favorable physicochemical characteristics of the oil allow it to be easily applied for various commercial applications. Although the oil is edible, its presence in the food industry is not very common and has instead entered the cosmetics industry's niche market. Some years ago, the prickly pear plant drew the research community's attention due to its potential versatile economic applications from the pericarp, pulp to its seeds, and its unique composition of several beneficial compounds (Piga, 2004; Ciriminna et al., 2017). This chapter is intended to provide insight into the published data on *Opuntia* seed oil, particularly their extraction methods and yields, physicochemical properties, fatty acid composition, tocopherol, sterol composition, and their potential application in the health and cosmetic sectors.



## 2 Extraction Methods and Yields

The extraction methods for obtaining *Opuntia* seed oil include cold pressing, organic solvents, supercritical carbon dioxide (scCO<sub>2</sub>), and ultrasound extractions. Table 33.1 summarizes reported extraction methods and their oil yields obtained from *Opuntia* species and their sampling locations. The *Opuntia* fruit is composed of about 2–12% seeds with an approximate weight of 67–216 g (El Kossori et al., 1998; Piga, 2004; Karabagias et al., 2020) and about 0.24 seeds/g fruit pulp (De Wit et al., 2016; Ciriminna et al., 2017). The general preparation method in obtaining the seeds for oil extraction include the following steps (Chougui et al., 2013; Ghazi et al., 2013; Ramírez-Moreno et al., 2017; Belviranlı et al., 2019; Regalado-Rentería et al., 2020):

1. Undamaged and ripe/mature *Opuntia* fruits are harvested and peeled manually,
2. Fruit is homogenized or liquefied to obtain the fruit pulp containing the seeds,
3. Fruit pulp is sieved or sifted to remove seeds from the pulp,
4. Seeds are then washed with water and dried (sun-dried/room temperature or drying at 60 °C until constant mass), and then
5. Seeds are grounded and used for oil extraction.

The oil yield from a species of *Opuntia* collected from different areas within a region can vary (Matthaüs & Özcan, 2011). Similarly, harvesting times have been shown to affect the oil content of *O. ficus-barbarica* A. Berger from Turkey (Al Juhaimi et al., 2020). Oil content ranged from 3.09% to 6.80% for sample collected from June to August (Al Juhaimi et al., 2020). The use of supercritical carbon dioxide (46.51 °C, 46.96 MPa, 10 kg CO<sub>2</sub>/h for 2.79 h) in the extraction of seed oil from *O. dillenii* Haw. resulted in an optimum yield of 6.65% (Liu et al., 2009). The ultrasound extraction method has also been applied to extract oil from *O. ficus indica*, Reyna variety, from Mexico (Ortega-Ortega et al., 2017a). The yield of oil was shown to be proportional to the amplitude level (Ortega-Ortega et al., 2017a). Generally, the oil yield of *Opuntia* species varies from 1% to 20% depending on species, cultivation/growing area, extraction methods, and harvesting times (Table 33.1). Cold pressing produces about 1 L oil from 800 to 1000 kg fruits or 25 kg seeds (Prakash & Sharma, 2014; Mule, 2016), while the use of organic solvents produces a higher oil yield. Green extraction technologies are more favored due to the adverse health and environmental issues associated with organic solvents (Koubaa et al., 2017). Oil extracted from *O. ficus-indica* from Tunisia was reported to be of better quality than the oil extracted with Soxhlet with *n*-hexane (Yeddes et al., 2012). The use of scCO<sub>2</sub> and cold pressing extraction technologies are preferred in terms of the oil's application in cosmetics and health products (Koubaa et al., 2017).

**Table 33.1** Extraction methods and yields of *Opuntia* seed oil (%)

Specie	Source of <i>Opuntia</i>	Extraction method	Yield <sup>a</sup> (%)	Reference
<i>O. ficus-indica</i> L.	Saudi Arabia	Soxhlet-petroleum ether	13.6	Sawaya and Khan (1982)
Tapón ( <i>Opuntia robusta</i> )	Mexico		20.0	Delgado and Pimienta-Barrios (1994) in: Pimienta-Barrios (1994)
Cascarón ( <i>Opuntia hyptiacantha</i> Weber)			16.2	
Bola de masa ( <i>Opuntia</i> sp.)			15.4	
Fafayuco ( <i>Opuntia</i> sp.)			14.4	
Redonda ( <i>Opuntia</i> sp.)			14.4	
Cardona ( <i>O. streptacantha</i> )			14.2	
Pachon ( <i>O. streptacantha</i> )			11.6	
Chapeada ( <i>Opuntia</i> sp.)			10.6	
Cristalina ( <i>Opuntia</i> sp.)			10.1	
Amarilla ( <i>O. ficus-indica</i> )			9.8	
Rojo-pelón ( <i>O. ficus-indica</i> )			8.4	
Burrona ( <i>Opuntia</i> sp.)			6.4	
<i>O. ficus-indica</i> L.	Italy	Hexane	8–9	Salvo et al. (2002)
<i>O. ficus-indica</i> L.	Turkey	Soxhlet-petroleum ether	54–69 g/kg	Coşkuner and Tekin (2003)
<i>O. ficus-indica</i> L.	Germany	Chloroform-methanol	98.8 g/kg	Ramadan and Mörsel (2003)
<i>O. ficus-indica</i> L.	Tunisia	Soxhlet-Hexane	11.8	El Mannoubi et al. (2009)

(continued)

**Table 33.1** (continued)

Specie	Source of <i>Opuntia</i>	Extraction method	Yield <sup>a</sup> (%)	Reference
<i>Opuntia dillenii</i> Haw.	China	SCCO <sub>2</sub>	6.65	Liu et al. (2009)
<i>Opuntia</i> sp.	South Africa	Chloroform-methanol		Labuschagne and Hugo (2010)
Roedtan			5.54	
Meyers			5.69	
Turpin			4.74	
Algerian			4.45	
Malta			5.43	
Zastron			3.91	
Morado			4.95	
Gymno Carpo			3.94	
Nudosa			5.16	
Fasicaulis			2.24	
Skinners Court			4.40	
<i>O. ficus-indica</i> L.	Turkey	Soxhlet-petroleum ether	5.0–14.4	Matthäus and Özcan (2011)
<i>O. ficus-indica</i> L.	Tunisia	Soxhlet-petroleum ether	4.4–6.9	Tlili et al. (2011)
<i>O. ficus-indica</i> L.	Turkey	Soxhlet-diethyl ether	5.0	Özcan and Al Juhaimi (2011)
<i>O. joconostle</i> , cv. Cuaresmeño	Mexico	Soxhlet	2.45	Morales et al. (2012)
<i>O. matudae</i> , cv Rosa			3.52	
<i>O. ficus-indica</i>	Algeria	Soxhlet	10.45 mg/100 g	Boukeloua et al. (2012)
<i>O. ficus-indica</i> L.	Morocco	Cold-pressed	6–7	Zine et al. (2013)
<i>O. elatior</i> (Mill.)	India	Soxhlet-Petroleum ether	13.6	Bhatt and Nagar (2013)
<i>O. dillenii</i>	Morocco	Hexane	5.7	Ghazi et al. (2013)
<i>O. ficus-indica</i>	Morocco	Hexane	5.1	Ghazi et al. (2013)
<i>O. ficus-indica</i>	Algeria	Soxhlet-hexane		Chougui et al. (2013)
Red			7.3	
Orange			7.7	
Yellow			9.3	
Green			8.4	

(continued)

**Table 33.1** (continued)

Specie	Source of <i>Opuntia</i>	Extraction method	Yield <sup>a</sup> (%)	Reference
<i>O. stricta</i> Haw.	Kenya	Petroleum ether	11.5	Kunyanga et al. (2014)
<i>O. ficus-indica</i>	Morocco	Soxhlet-hexane	9.16 (Seed diameter > 2.25 mm)	Taoufik et al. (2015)
<i>Opuntia stricta</i> , <i>Haworth variety</i> (Haw.)	Tunisia	Soxhlet-hexane	235 g/kg	Koubaa et al. (2017)
		Hexane	49%	
		SCCO <sub>2</sub>	49.9%	
<i>O. ficus-indica</i> (Algerian, Gymno Carpo, Meyers, Morado, Nudosa, Roedtan, Sicilian Indian Fig, Skinners Court, Tormentosa, Turpin, Van As, and Zastron)	South Africa	Chloroform and methanol	5.8–6.7% between locations	de Wit et al. (2016)
			5.8–6.67% between seasons	
			3.45–8.23% among cultivars	
			5.42–6.67% for cultivar × location × season interaction	
<i>O. dillenii</i>	Madagascar	Petroleum ether-ANKOM XT 1 5 extraction system	7.04	Hänke et al. (2018)
<i>O. stricta var. stricta</i>	Madagascar	Petroleum ether—ANKOM XT 1 5 extraction system	8.8	Hänke et al. (2018)
<i>O. albicarpa</i>	Mexico	Hexane	11.83	Ramírez-Moreno et al. (2017)
		Ethanol	10.13	
		Ethyl acetate	10.6	
<i>O. ficus-indica</i>	Mexico	Hexane	6.69	Ramírez-Moreno et al. (2017)
		Ethanol	5.11	
		Ethyl acetate	3.81	
<i>O. ficus-indica</i> (Reyna)	Mexico	Ultrasound	3.75–6.00	Ortega-Ortega et al. (2017a, b)
		Soxhlet-hexane	7–8	
		Maceration	5–6	
<i>O. ficus-indica</i> Algerian Meyers Morado Nudosa Tormentosa <i>O. robusta</i> Monterey Robusta	South Africa	Chloroform-methanol	7.82	de Wit et al. (2017a)
			8.00	
			8.04	
			6.85	
			8.09	
			5.65	
			6.46	

(continued)

**Table 33.1** (continued)

Specie	Source of <i>Opuntia</i>	Extraction method	Yield <sup>a</sup> (%)	Reference
<i>O. ficus-indica</i> (40 cultivars)	South Africa	Chloroform-methanol	6.24	De Wit et al. (2017b)
<i>O. robusta</i> (Robusta and Monterey).				
<i>O. ficus-indica</i> L.	Turkey	Soxhlet-petroleum ether	5.34–7.67	Belviranlı et al. (2019)
<i>Opuntia ficus-indica</i> Sanguigna and the yellow fruits of <i>O. ficus-indica</i> Surfarina varieties (voucher MB101/2017 and MB 102/2017, <i>O. ficus-indica</i> Sanguigna)	Italy	Soxhlet-hexane	9.3	Loizzo et al. (2019)
		USM	5.4	
		Soxhlet-hexane	9.5	
<i>O. ficus-indica</i> Surfarina		USM	5.6	
<i>O. dillenii</i>	Iraq	Hydro-distillation	6.5	Alsaad et al. (2019)
Amarilla Monteza	Mexico	Cold-pressed	1.66	Regalado-Rentería et al. (2020)
( <i>O. megacantha</i> )		MP	7.63	
Blanca		Cold-pressed	1.19	
( <i>O. albicarpa</i> )		MP	8.72	
Cardona		Cold-pressed	2.52	
( <i>O. streptacantha</i> )		MP	11.64	
Charola		Cold-pressed	0.51	
( <i>O. streptacantha</i> )		MP	10.55	
Pico Chulo		Cold-pressed	–	
( <i>O. megacantha</i> )		MP	6.16	
Rojo Tapón		Cold-pressed	6.08	
( <i>O. robusta</i> )		MP	15.54	
Tapona		Cold-pressed	5.71	
( <i>O. robusta</i> )		MP	14.54	
Xoconostle		Cold-pressed	1.65	
( <i>O. matudae</i> )	MP	9.68		
<i>O. ficus-indica</i> (L.)	Morocco	n-hexane	8.86	Gharby et al. (2020)
		2-MeO	9.55	
<i>O. ficus-barbarica</i> A. Berger	Turkey	Soxhlet-petroleum ether	3.09–6.80	Al Juhaimi et al. (2020)

SCCO<sub>2</sub> supercritical carbon dioxide, USM ultrasound-assisted maceration, MP Maceration-percolation, 2-MeO 2-methyloxolane

<sup>a</sup>%, unless otherwise stated within Table; Yield data ranges reported can be indicative of cultivars for the same species, different sample collection sites, harvesting periods and extraction methods

### 3 Physicochemical Properties

The most-reported physicochemical properties were for *O. ficus-indica* seed oil, while the physicochemical properties of *O. elatior* (Mill.) (Bhatt & Nagar, 2013) and *O. robusta* seed oil (De Wit et al., 2017a) have also been reported (Table 33.2). *Opuntia* seed oil is an edible oil, with reported low toxicity (Boukeloua et al., 2012), which is light green to yellow (Sawaya & Khan, 1982; Moutkane, 2015) and is a liquid at room temperature (El Mannoubi et al., 2009). The seed oil's refractive index and density range from 1.4596 to 1.4831 and 0.904–0.907, respectively (Table 33.2). The iodine values range from 111 to 132 g I<sub>2</sub>/100 g oil, which refers to the high degree of unsaturation in the *Opuntia* seed oil (Table 33.3). A significant variation in peroxide values of the *Opuntia* seed oil obtained from different species and their origin have been reported (Table 33.2). The peroxide values recorded are low and indicate the oil's oxidative stability and quality (Table 33.2). The seed oil has low acid values, and saponification values ranging from 173 to 222 mg KOH/g oil. The unsaponifiable matter ranges from 1.19% to 2.65%. The physicochemical properties have been shown to vary among *Opuntia* seed oils obtained from different cultivars (De Wit et al., 2017a). *O. ficus-indica* seed oil from Greece has been reported (Karabagias et al., 2020) to be rich in aroma due to the presence of a wide range of organic compounds and, in particular, volatile compounds. The aroma is described to be of a floral and fruity nature (Moutkane, 2015). The oil has been reported to be stable for about 18 months, if kept under prescribed storage conditions (Moutkane, 2015).

### 4 Fatty Acid Composition

The main species for which the fatty acid composition of the seed oil has been reported are *O. ficus-indica*; others include *O. streptacantha*, *O. robusta*, *O. boldin-ghii*, *O. joconostle*, *O. matudae*, *O. elatior*, *O. dillenii*, *O. albicarpa*, *O. ficus-barbarica*, *O. aequatorialis*, *O. leucotricha* and *O. megacantha* (Table 33.3). The dominant fatty acid found in the *Opuntia* seed oil is linoleic acid in a concentration range of 56–77%, followed by palmitic acid (9–23%) and oleic acid (2–29%) (Table 33.3). Other fatty acids found in minor concentrations are myristic acid, palmitoleic acid, stearic acid, linolenic acid, and behenic acid, depending on the origin of the species and the specific species cultivar of *Opuntia*. The fatty acid composition of *Opuntia* seed oil is similar to that of the sunflower and grape seed oils (Labuschagne & Hugo, 2010; El-Mostafa et al., 2014). The content of linoleic acid in *Opuntia* seed oil is higher than that reported for argan oil (El-Mostafa et al., 2014). The fatty acid composition in *Opuntia* species collected from different areas within a region can vary (Matthäus & Özcan, 2011; Ramadan & Mörsel, 2003). Similarly, harvesting times have been shown to affect the oil content of *O. ficus-barbarica* A. Berger from Turkey (Al Juhaimi et al., 2020). Plant genetics, soil

**Table 33.2** Physicochemical characteristics of *Opuntia* seed oil

Specie	Origin	Refractive index	Iodine number (g I <sub>2</sub> /100 g)	Saponification number	Acid value (% oleic)	Unsaponifiable matter (%)	Peroxide value (meq O <sub>2</sub> /kg)	K		Density	Reference
								K <sub>232</sub>	K <sub>270</sub>		
<i>O. ficus-indica</i>	Saudi Arabia	1.4596	119	222	0.84	1.96					Sawaya and Khan (1982)
	Italy		106		2.5		10	3.15			Salvo et al. (2002)
				107	173	1.27		1.46	0.22	0.904	
	Turkey	1.4831		181	1.41		1.63		0.907		Özcan and Al Juhaimi (2011)
Algeria	0.909		93		1.82	1.476			2.04		Boukeloua et al. (2012)
		1.4610	131	187	0.56	1.19	3.5	1.72	0.906		Zine et al. (2013)
Morocco	1.4666–1.4669		111–124				9.50–18.02	0.31			De Wit et al. (2017a)
	1.470						12.0				Brahmi et al. (2020)
Morocco			132		1.26–3.02		3.5–8.6	2.75–3.25			Gharby et al. (2020)
								0.51–2.11			
<i>O. elatior</i>	India		111	192	1.64	2.65					Bhatt and Nagar (2013)
<i>O. robusta</i>	South Africa	1.4675–1.4676	122–127				20–24				de Wit et al. (2017a)

Note: Data ranges reported can be indicative of cultivars for the same species, different sample collection sites, harvesting periods, and extraction methods

**Table 33.3** Fatty acid composition (%) of *Opuntia* seed oil

Specie	Source	C16:0	C18:0	C18:1	C18:2	References
<i>O. ficus-indica</i>	Saudi Arabia, Mexico, Germany, Turkey, Tunisia, Algeria, Morocco, South Africa, Italy	10–20	0.15–5.8	8.8–27	49–77	Sawaya and Khan (1982), Delgado and Pimienta-Barrios (1994) in: Pimienta-Barrios (1994), Ramadan and Mörsel (2003), Coşkuner and Tekin (2003), El Mannoubi et al. (2009), Matthaüs and Özcan (2011), Özcan and Al Juhaimi (2011), Tlili et al. (2011), El Finti et al. (2013), Zine et al. (2013), Ghazi et al. (2013), Chougui et al. (2013), Taoufik et al. (2015), Ramírez-Moreno et al. (2017), R'bia et al. (2017), de Wit et al. (2017a), de Wit et al. (2017b), Belviranlı et al. (2019), Loizzo et al. (2019), Gharby et al. (2020), El Kharrassi et al. (2020)
<i>O. streptacantha</i>	Mexico	4.7–14	1.1–3.9	14–20	61–80	Delgado and Pimienta-Barrios (1994) in: Pimienta-Barrios (1994), Regalado-Rentería et al. (2020)
<i>O. boldinghii</i>	Venezuela	10	3	18	67	García Pantaleón et al. (2009)
<i>O. joconostle</i>	Mexico	12.4	3.3	9.7	72.5	Morales et al. (2012)
<i>O. matudae</i>	Mexico	0.7–9.4	0.3–2	7.8–15.2	79.2–83.8	Morales et al. (2012), Regalado-Rentería et al. (2020)
<i>O. elatior</i>	India	12.2	3.5	16.9	65.8	Bhatt and Nagar (2013)
<i>O. dillenii</i>	Iraq, Morocco	14–15.1	3–7.51		72.9–80	Ghazi et al. (2013), Alsaad et al. (2019)
<i>O. albicarpa</i>	Mexico	0.3–12	0.3–3	16–20.7	67–78.6	Ramírez-Moreno et al. (2017), Regalado-Rentería et al. (2020)
<i>O. robusta</i>	Mexico, South Africa	0.57–16	0.34–2.4	11.2–22	60–86	Delgado and Pimienta-Barrios (1994) in: Pimienta-Barrios (1994), de Wit et al. (2017a), Regalado-Rentería et al. (2020)
<i>O. aequatorialis</i>	Morocco	12.2	3.5	21.5	60.9	El Kharrassi et al. (2020)
<i>O. leucotricha</i>	Morocco	12	3.6	21.1	61.6	El Kharrassi et al. (2020)
<i>O. megacantha</i>	Mexico, Morocco	0.3–12	0.5–3.5	13.2–20.8	62–79	Regalado-Rentería et al. (2020), El Kharrassi et al. (2020)
<i>O. ficus-barbarica</i>	Turkey	10.7–22.6	3.9–9.2	2–28.5	0.76–57.5	Al Juhaimi et al. (2020)
<i>Opuntia</i> sp.	Mexico, South Africa	9–16	0.7–4.01	12–17	61–77	Delgado and Pimienta-Barrios (1994) in: Pimienta-Barrios (1994), Labuschagne and Hugo (2010)

Note: Concentration ranges reported can indicate different cultivars for the same species, different sample collection sites, harvesting periods, and extraction methods



conditions, and climate conditions influence the composition of fatty acids of *Opuntia* seeds oil (Matthäus & Özcan, 2011; Ramadan & Mörsel, 2003). The fatty acid composition of seed oil from *O. ficus-indica*, Sanguigna (red) variety, and *O. ficus-indica*, Surfarina (yellow) variety of Italy is not affected by the extraction procedure used such as Soxhlet (*n*-hexane) and ultrasound-assisted maceration procedure (Loizzo et al., 2019). *O. megacantha* Salm-dyck seed oil has been reported to contain a higher linoleic acid content as compared to argan and olive oil (El Kharrassi et al., 2018). The eicosadienoic acid (C20:2, 1.7%), an *omega*-6 fatty acid, has been reported by Bhatt and Nagar (2013) to be contained in *O. elatior* (Mill.) from India. *O. ficus-indica* seed oil from Tunisia has been found (El Mannoubi et al., 2009) to contain 5% of vaccenic acid (18:1*n*-7).

## 5 Tocopherol and Sterol Composition

The most common species for which the tocopherol composition of *Opuntia*'s seed oil has been reported is *O. ficus-indica* from markets such as Germany, Tunisia, Turkey, Morocco, and Italy. At the same time, other species also include *O. dillenii* (Morocco), *O. megacantha* (Mexico), *O. albicarpa* (Mexico), *O. streptacantha*, *O. robusta* (Mexico), *O. matudae* (Mexico), *O. aequatorialis* (Morocco), and *O. leucotricha* (Morocco). Table 33.4 provides an overview of the tocopherol composition of the seed oil from various species of *Opuntia*. The reported dominant sterol is  $\gamma$ -tocopherol, while  $\alpha$ ,  $\beta$ ,  $\delta$ -tocopherol have been reported to be present in some *Opuntia* seed oils. Tocopherols such as  $\alpha$ -tocopherol,  $\alpha$ -tocopherol,  $\delta$ -tocopherol,  $\alpha$ -tocotrienol,  $\alpha$ -tocotrienol, plastochromanol-8,  $\gamma$ -tocotrienol, and  $\delta$ -tocotrienol were not detected in *O. ficus-indica* L. seed oil from Turkey (Matthäus & Özcan, 2011). The geographical location affects the composition and concentration of tocopherol in *Opuntia* seed oil (Matthäus & Özcan, 2011; Taoufik et al., 2015). The  $\gamma$ -tocopherol content of seed oil from *O. ficus-indica*, Sanguigna (red) variety and *O. ficus-indica*, Surfarina (yellow) variety of Italy is reported to be affected by the extraction procedure, wherein Soxhlet (*n*-hexane) extracted a higher content as compared to the ultrasound-assisted maceration procedure (Loizzo et al., 2019). *O. megacantha* Salm-dyck seed oil contains mainly  $\beta$ -tocopherol and  $\gamma$ -tocopherol (El Kharrassi et al., 2018). Various sterols are found in *Opuntia* seed oil, with  $\alpha$ -sitosterol as the most dominant sterol (Table 33.5). The sterol, fucosterol, has been detected in the seed oil obtained from *O. dillenii* from Morocco (Ghazi et al., 2013). Stigmastanol (47 mg/100 g) has been found in the seed oil of *O. ficus-indica* from Algeria (Brahmi et al., 2020). Regalado-Rentería et al. (2020) reported the presence of the squalene in the seed oil of *O. megacantha*, *O. albicarpa*, *O. streptacantha*, *O. robusta*, and *O. matudae* from Mexico.

**Table 33.4** Tocopherol of *Opuntia* seed oil (mg/100 g)<sup>a</sup>

Specie	Source	$\alpha$ -tocopherol	$\alpha$ -tocopherol	$\gamma$ -tocopherol	$\delta$ -tocopherol	References
<i>O. ficus-indica</i>	Germany, Tunisia, Turkey, Morocco, Italy	1–5.6	1.2	15.3–85.6	0.5–8.26	Ramadan and Mörsef (2003), El Mannoubi et al. (2009), Matthäus and Özcan (2011), Zine et al. (2013), Ghazi et al. (2013), Ghazi et al. (2013), Taoufik et al. (2015), Loizzo et al. (2019), El Kharrassi et al. (2020), Gharby et al. (2020)
<i>O. megacantha</i>	Mexico			118–139		Regalado-Rentería et al. (2020), El Kharrassi et al. (2020)
<i>O. albicarpa</i>	Mexico			136–156		Regalado-Rentería et al. (2020)
<i>O. streptacantha</i>	Mexico			134–406		Regalado-Rentería et al. (2020)
<i>O. robusta</i>	Mexico			150–1386		Regalado-Rentería et al. (2020)
<i>O. matudae</i>	Mexico			119–373		Regalado-Rentería et al. (2020)
<i>O. aequatorialis</i>	Morocco	1.3%		98.3%	0.39%	El Kharrassi et al. (2020)
<i>O. leucotricha</i>	Morocco	0.96%		98.7%	0.38%	El Kharrassi et al. (2020)
<i>O. dillenii</i>	Morocco			0.29%		Ghazi et al. (2013)

Note: Concentration ranges reported can indicate cultivars for the same species, different sample collection sites, and extraction methods

<sup>a</sup>mg/100 g unless otherwise stated in the Table

## 6 Health and Cosmetic Applications

*Opuntia* seed oil has been reported to have a wide range of health applications (Table 33.6), which include antimicrobial and antifungal activities, analgesic and anti-inflammatory effects,  $\alpha$ -glucosidase inhibitory activity and cytotoxicity against certain cancer cell lines from the human origin (Ramírez-Moreno et al., 2017; Villacís-Chiriboga et al., 2020). Table 33.5 provides a summary of the health benefits of *Opuntia* seed oil and their potential industrial applications. The seeds of prickly pear have been reported to have antioxidant activity towards lipid peroxidation (González-Stuart & Rivera, 2019). The seeds of *O. ficus-indica* are also reported to be used in traditional medicine (Boukeloua et al., 2012). The antioxidant ability and hypoglycemic effect of seed oils from *O. ficus-indica*, Sanguigna (red) variety, and *O. ficus-indica*, Surfarina (yellow) variety of Italy have been demonstrated by Loizzo et al. (2019). *O. albicarpa* and *O. ficus-indica* from Mexico are useful as antimicrobials and antioxidants (Ramírez-Moreno et al., 2017). The presence of phenolic compounds and tocopherols in the oil contributes to the good antioxidant activity because these compounds are capable of influencing cellular responses to various oxidative stresses *via* modulating signal-transduction pathways (Eckardt, 2008; Maeda et al., 2008). The consumption of oil has been associated with a reduced risk of developing cardiovascular, inflammatory, and autoimmune diseases (Chahdoura et al., 2017). *O. ficus-indica* seed oil was reported to have hypocholesterolemic and hypolipidemic activities (Ennouri et al., 2007). Cactus pear seed oil has also been reported to have the ability to prevent alloxan-induced-diabetes by quenching free radicals produced by alloxan and inhibiting tissue injuries in pancreatic  $\beta$  cells (Berraouan et al., 2015). Cold pressed *Opuntia* seeds oil extracted from *O. ficus-indica* (Morocco) is useful in treating diabetes mellitus (Berraouan et al., 2015). The *Opuntia* seed oil has been applied in encapsulating vitamin A towards the use as a topical delivery system of vitamin A (Al Zahabi et al., 2019). The dominant presence of linoleic acid at a high concentration in *Opuntia* seed oil (Table 33.3) also contributes to the oil's health benefits (Soel et al., 2007). The health benefits and antioxidant potential of phenolic compounds is well reported. Chbani et al. (2020) have suggested developing a phenolic compound composition fingerprint to detect adulteration and authenticity of the *Opuntia* seed oil. The dominant phenolic compounds in the seed oil of *O. ficus-indica* from Morocco are ferulaldehyde, vanillin, and syringaldehyde. Roasting of seeds before oil extraction resulted in differences in phenolic compounds' composition, except vanillin (Chbani et al., 2020).

*Opuntia* seed oil is a beneficial oil widely advertised for skincare applications (Argan Oil Direct, 2020). It is marketed as oil with good hydration potential, anti-aging and antioxidant potential, improved skin elasticity, and the ability to reduce skin redness and pigmentation (Joslin, 2017; Opuntia Luxury Oils, 2020; Argan Oil Direct, 2020). The oil has a ratio of 3:1 of linoleic to oleic acid, making it suitable for cosmetic applications (Opuntia Luxury oils). The oil's antimicrobial ability makes it suitable to develop skincare products to treat acne (Healthline, 2020). The oil is reported to contain vitamin K<sub>1</sub> (0.53 g/kg) that provides the oil with the ability

**Table 33.5** Sterol compositions of *Opuntia* seed oil (mg/100 g)<sup>a</sup>

Specie	Source	Campesterol	Stigmasterol	$\alpha$ -sitosterol	Squalene	Cholesterol	$\Delta^5$ -Avenasterol	$\Delta^7$ -Avenasterol	$\Delta^7$ -Stigmasterol	References
<i>O. ficus-indica</i>	Germany, Tunisia, Turkey, Morocco, Italy	11.6–21.7	3.3–11.3	76–387		1.5–1.8	4–4.4	2–2.17	1.8–2.2	Ramadan and Mörsel (2003), El Mannoubi et al. (2009), Matthäus and Özcan (2011), Zine et al. (2013), Ghazi et al. (2013), Taoufik et al. (2015), Loizzo et al. (2019), El Kharrassi et al. (2020), Gharby et al. (2020), Brahmi et al. (2020)
<i>O. megacantha</i>	Mexico	10.3% <sup>b</sup>	1.48% <sup>b</sup>	456–721	245–1255	1% <sup>b</sup>	5.2% <sup>b</sup>	1% <sup>b</sup>	0.56% <sup>b</sup>	Regalado-Rentería et al. (2020), El Kharrassi et al. (2020)
				74.6% <sup>b</sup>						
<i>O. albicarpa</i>	Mexico			608–777						Regalado-Rentería et al. (2020)

Specie	Source	Campesterol	Stigmasterol	$\alpha$ -sitosterol	Squalene	Cholesterol	$\Delta^5$ -Avenasterol	$\Delta^2$ -Avenasterol	$\Delta^7$ -Stigmasterol	References
<i>O. streptacantha</i>	Mexico			601–710						Regalado-Rentería et al. (2020)
<i>O. robusta</i>	Mexico			507–730						Regalado-Rentería et al. (2020)
<i>O. mattudae</i>	Mexico			581–641						Regalado-Rentería et al. (2020)
<i>O. aequatorialis</i>	Morocco	10.2%	1.1%	76.2%		0.83%	5.4%	1.1%	0.89%	El Kharrassi et al. (2020)
<i>O. leucotricha</i>	Morocco	11.2%	1.6%	72.7%		1.01%	5.4%	1.3%	0.59%	El Kharrassi et al. (2020)
<i>O. dillenii</i>	Morocco	0.51%		2.8%						Ghazi et al. (2013)

Note: Concentration ranges reported can indicate cultivars for the same species, different sample collection sites, harvesting periods, and extraction methods <sup>a</sup>mg/100 g unless otherwise stated in the Table; <sup>b</sup>El Kharrassi et al. (2020)

**Table 33.6** Health applications of *Opuntia* seed oil

<i>Opuntia</i> specie	Health benefit	Industrial application(s)	Reference(s)
<i>O. ficus-indica</i>	Antimicrobial and antioxidant, decrease circulating cholesterol and LDL-cholesterol, lipid peroxidation, hypolipidemic effect, natural source of edible oil containing essential fatty acids, modulation of cholesterol metabolism inhibition of tissue injuries in pancreatic $\beta$ -cells, hypoglycemic effect, hypolipidemic and hypocholesterolemic	Food, cosmetic, pharmaceutical, cosmeceuticals (Topical lipid delivery system), wound healing, nutraceutical, natural food colorants	Stintzing et al. (2005), Ennouri et al. (2005), Ennouri et al. (2006), Ennouri et al. (2007), Özcan and Al Juhaimi (2011), Chougui et al. (2013), Battermann and Thomas (2014), El Kharrassi et al. (2014), Berraouan et al. (2014, 2015) Ramírez-Moreno et al. (2017), Ortega-Ortega et al. (2017b), R'bia et al. (2017), Khémiri et al. (2019), Al Zahabi et al. (2019), Belviranlı et al. (2019), Loizzo et al. (2019), Karabagias et al. (2020), Brahmī et al. (2020)
<i>O. albicarpa</i>	Antimicrobial and antioxidant, Anti-UV radiation	Food, cosmetic, pharmaceutical, cosmeceutical	Ramírez-Moreno et al. (2017), Regalado-Rentería et al. (2020)
<i>O. macrorhiza</i>	Antioxidant, cytotoxicity, $\alpha$ -glucosidase inhibition, antimicrobial, analgesic effect, and anti-inflammatory properties	Functional food	Chahdoura et al. (2017)
<i>O. dillenii</i>	Antioxidant activity, lowering cholesterol	Food, nutraceutical	Liu et al. (2009), Alsaad et al. (2019)
<i>O. robusta</i>	Antioxidant activity, anti-UV radiation	Natural food colorants, Cosmeceutical	Stintzing et al. (2005), Regalado-Rentería et al. (2020)
<i>O. megacantha</i>	Anti-UV radiation	Cosmeceutical	Regalado-Rentería et al. (2020)
<i>O. matudae</i>			
<i>O. streptacantha</i>			
<i>O. aequatorialis</i>	Antioxidant activity	Functional foods	El Kharrassi et al. (2020)
<i>O. leucotricha</i>			
<i>Opuntia</i> spp.	Antioxidant, hypoglycemic, antineoplastic, antibacterial, and antifungal activity, Positive effects on mitochondrial activity	Food, cosmetic, pharmaceutical	Ennouri et al. (2006, 2007), Badreddine et al. (2015), Aruwa et al. (2018)
<i>O. stricta</i>	antioxidant and antibacterial activities	Food and pharmaceutical	Koubaa et al. (2017)

to reduce dark/under-eye circles and spider veins (Ramadan & Mörsel, 2003; Argan Oil Direct, 2020; Daya, 2020). The oil is non-greasy and easily absorbed onto the skin. While the direct application of *Opuntia* seed oil on the skin is known, the oil is also used as a carrier oil to produce other cosmetic products (Healthline, 2020). *Opuntia* seed oil is also applied in hair products (Argan Oil Direct, 2020), with some treatments have been patented (Battermann & Thomas, 2014).

## 7 Conclusion

*Opuntia* seed oil can be obtained from a wide range of different species and cultivars of *Opuntia* worldwide. Harvesting times, geographical locations, and species type or cultivar affect the biochemistry of *Opuntia* seed oil. In terms of its chemical composition, it is considered safe with various health benefits. The oil is actively promoted in the cosmetics industry, but there is a great potential for developing cosmeceuticals and nutraceuticals from *Opuntia* seed oil.

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# Chapter 34

## Prickly Pear (*Opuntia ficus indica*) Processing by Extrusion-Cooking



Martha Graciela Ruiz-Gutiérrez and Armando Quintero-Ramos

**Abstract** Extrusion cooking is a process that involves feeding, mixing, and cooking material at a high temperature for a short time to obtain a wide variety of products. The final product characteristics depend mainly on variables such as mixing speed, barrel temperature, feed moisture content, and system pressure. One of the most critical variables that influence the characteristics and quality of the product is the mixture's composition. Several ingredients have been used in mixture formulation to improve nutritional quality and physical characteristics that increase the extruded products' acceptability. Among the ingredients are grains, vegetables, and fruits applied as extracts, concentrates or powders. A fruit interesting for its composition is a prickly pear, with properties that can be used to develop novel and/or functional food or a clean label product. The red prickly pear contains flavonoid-type phenolics and betalain pigments, which give it an intense red-purple color that can be used to color food. These constituents give the fruit functional properties; however, both are heat-labile compounds, and therefore, their use in the formulation of extruded products must be tested. The addition of prickly pear reduces the extrudates' density and expansion and the water absorption capacity as the prickly pear content increases. In contrast, characteristics such as bulk density, texture (hardness and crispness), and color parameters increase as the prickly pear content increases. Although the addition of prickly pear impacts the most physical characteristics, such as the color and texture parameters of extrudates, the addition of a moderate amount of prickly pear powder in the formulation of extruded cereals is accepted by consumers, especially for the resulting color in the products. During extrusion cooking, pigments are lost due to temperature, wherein betaxanthin is more sensitive to the increase in temperature. Other compounds affected during extrusion cooking are phenolics, although some studies have shown that the specific content of flavonoids remains similar to the raw mixture's content. Likewise, by decreasing the betalain and phenolics' content, extrusion cooking decreases the antioxidant activity they provide. Although the level of reduction of the compounds depends on

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the specific conditions under which the process is carried out, the use of red prickly pear, as pulp or powder, in the formulation of extruded foods can allow the elimination of synthetic dyes from cereals or snacks, improving the functional properties of food. This chapter addresses essential aspects of extrusion cooking and describes the effects of the main process variables on bioactive compounds' stability and other physicochemical properties in extruded products based on prickly pear.

**Keywords** Functional properties · Opuntia · Prickly pear · Betalains · Bulk density · Texture

#### Abbreviations

<i>a</i>	Blueness-redness tendency
$A_0$	is the frequency factor
<i>b</i>	Greenness-Yellowness tendency
°C	Centigrade grades
<i>Ea</i>	Activation energy
HT	High temperature
HT-ST	High-temperature—short-time
J	Joule
<i>k</i>	Degradation constant
K	Kelvin
<i>L</i>	Luminosity
L/D	Length/diameter ratio
<i>R</i>	Gas constant
<i>RTD</i>	Residence time distribution
s	Seconds
ST	Short time
T	Temperature
$tr_m$	Time medium of residence

## 1 Extrusion-Cooking Process

Extrusion-cooking technology is used to produce a wide variety of food products, such as breakfast cereals, snack food, pasta, baby food, textured vegetable proteins, animal food, meat analogs, energy bars, filled products, and instant flours. Extrusion cooking can be described as a continuous process in which raw material is plasticized to form a fluid melt in a barrel due to high temperature, pressure, and shear stress, allowing the material to be conveyed and forced to flow through a specific shape (Harper & Clark, 1979). Through this process, the materials fed are transported in a chamber through the use of one or two screws, during which they are subjected to heating caused by the addition of heat or by the friction itself generated

during transport, transforming into a semifluid paste subject to high temperatures and pressure, which is forced to pass through the given extruder, leading to the expansion of the extruded product (Ruiz-Gutiérrez et al., 2018). This process is based on high-temperature—short-time (HT-ST) thermal processes. The temperature is usually over 100 °C, and the processing time depends on the extruder type (simple or twin screw) and the length of the equipment, specifically the barrel (Guy & Horne, 1998). The process can be summarized by three main stages (Fig. 34.1) including feeding (mixing and preconditioning), mixing and cooking (transport and heating inside the barrel), and expansion (discharge from the end of the barrel).

According to the process described, extrusion technology involves applying thermal and mechanical energy to transport and cook the material through simple or rotating helical screws. Because of the high pressures, physical and chemical changes occur in the material fed. The main chemical and structural transformations in the feed material during the extrusion-cooking process are the gelatinization of starch, denaturation of proteins, and complexes between amylose and lipids. The main ingredients used in extrusion cooking are cereals, starches, and vegetable proteins. However, other components are used to contribute to the formulation of nutritious and functional food, such as vitamins, pigments, and antioxidants. Due to the heat-sensitive nature of these types of components, the degradation and loss of these compounds is the major transformation that occurs during extrusion cooking (Larrea et al., 2005; Camire et al., 2007; Ilo & Berghofer, 1999). These transformations depend on the conditions under which the extrusion-firing process is carried out, mainly the barrel temperature, the humidity of the feed mix, and the speed, type, and configuration of the screw (Kumar et al., 2008).

The specific conditions of each extrusion process, such as the moisture of the feed, the type of extruder, the temperature of the barrel, and the speed of the screw, directly affect the composition of the final product. Some studies have reported the effect of extrusion-cooking conditions on specific compounds:

- **Moisture content.** The retention percentage of some vitamin contents, such as vitamin E, is not affected by the mixture's moisture content (Anderson & Sunderland, 2002). However, some studies show that vitamin C is affected by



**Fig. 34.1** Extrusion-cooking stages

feed moisture content, decreasing its stability, probably because water helps hydrolyze the vitamin's phosphate molecule (Plunkett & Ainsworth, 2007). For components such as astaxanthin, increasing the feed moisture content increases the loss of this carotenoid (Anderson & Sunderland, 2002).

- **Extruder.** The type of extruder has an essential effect on the retention of bioactive components, among which are the B group of vitamins (riboflavin, pyridoxine, niacin, and thiamine) that are present in cereals (i.e., corn, and oats, etc.), since if long-barrel extruders are used, very low retention (20%) has been found. If short barrels are used, retention up to 62% can be achieved for less stable vitamins. The above is related to the temperature and the extrusion time, showing that HT-ST is better than low temperature-longer time processing (Athar et al., 2006).
- **Barrel temperature.** Increasing the extruder barrel's temperature has not been shown to decrease vitamins of group B (Athar et al., 2006), even when they are highly thermolabile. However, the loss of other vitamins, such as ascorbic acid (vitamin C), at screw speeds less than 200 rpm (Plunkett & Ainsworth, 2007) has been reported. However, there were no significant losses in antioxidant activity at very high screw speeds at 170 °C (Şensoy et al., 2006). Additionally, in anthocyanins, including glycosides, galactosides, cyanidin arabinosides, and peonidin, a decrease in concentration with increasing barrel temperature has been shown, with cyanidin 3-arabinoside and peonidin 3-arabinoside being the most affected. The maximum retention of the components occurs with intermediate percentages (30%) of bagasse, probably due to the starch's protective effect on the sensitive components.
- On the other hand, flavanols, including glycosides of myricetin and quercetin, as well as aglycones, are more stable against heat, and increases of 30–34% have been found after the extrusion process; however, another possible explanation for this effect is that after extrusion, the extraction of these components is higher due to the disruption of the bagasse matrix during extrusion. Concerning flavonoids, which include procyanidins, an increase in the concentration of monomers and dimers has been reported, in addition to a notable decrease in trimers. This behavior is because, during the extrusion process, there is a breaking of phenolic compounds' covalent bonds. This behavior is important since low molecular weight procyanidins are easier for the body to assimilate (White et al., 2010; Khanal et al., 2009). The use of high temperatures during extrusion produces a decrease in the products obtained' antioxidant capacity, probably due to the Maillard reactions produced since there is no correlation between the concentration of phenolics and the antioxidant capacity. At the same time, there is an increase in the darkening of products with increasing temperature (White et al., 2010). The content of betalains, including both betacyanins and betaxanthins, is reduced by temperature action; however, betaxanthins are more susceptible to increases in temperature. Increasing the temperature reduces the content, but when the temperature is very high, the material flow characteristics reduce the exposure time, preventing further degradation (Ruiz-Gutiérrez et al., 2015).



- **Screw speed.** It has been shown that increasing the speed of the extruder screw up to 300 rpm causes an increase in the retention of vitamins (56–79%) such as ascorbic acid because the retention time in the extruder is decreased (Plunkett & Ainsworth, 2007); however, riboflavin is lost due to the increased screw speed and shear stress (Athar et al., 2006). It has also been found that nonsignificant losses in antioxidant activity can occur at very high screw speeds, 500 rpm (Şensoy et al., 2006).

## 2 Ingredients for Extrusion Cooking

The basis of the formulation of the raw material's main components to obtain extruded food are carbohydrates, proteins, and lipids, but the current trend in food development is to obtain functional products. For this reason, studies have focused on obtaining functional products by adding legumes (Anton et al., 2009), cereals (Repo-Carrasco-Valencia et al., 2009), fruits (Khanal et al., 2009; Yağcı & Göğüş, 2008), fruit extracts (Hirth et al., 2014) and vegetables (Stojceska et al., 2008). Through these studies, it was found that the extrusion-cooking process can cause decreases in the concentration of compounds with antioxidant activity, such as carotenoids, anthocyanins, and flavanols. Among them are L-ascorbic acid (Plunkett & Ainsworth, 2007), vitamin E, and astaxanthin, mainly due to the increase in the temperature of the process (Anderson & Sunderland, 2002). However, other studies reported that during extrusion, there is an increase in some components such as free phenolic acids due to the conversion of types of phenols (Ortiz-Cruz et al., 2020; Zieliński et al., 2001), as well as an increase in total phenolic compounds and antioxidant capacity due to the formation of new compounds generated by the Maillard reaction that rise antioxidant activity (Stojceska et al., 2009; Camire et al., 2007; Pokorny & Schmidt, 2006).

Camire et al. (2007) studied the use of fruit powders (blueberries, grapes, and raspberries) to pigment extruded cereals. Although the cereals obtained had good color, the anthocyanin, and phenolic compound content decreased 10 times. Other studies reported the use of powders in the extrusion process to improve component retention. Yuliani et al. (2006) used encapsulated D-limonene powder in the extrusion-cooking process and reported higher retention, concluding that this was due to the formation of inclusion complexes with starch.

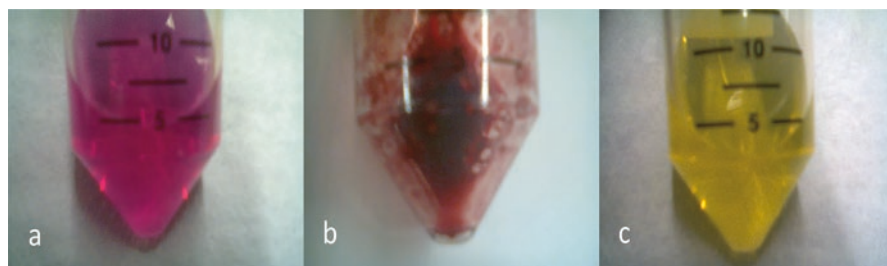
## 3 Prickly Pear Characteristics

The red prickly pear (*Opuntia ficus indica*) is a fruit of the cactus family that has a diversity of shapes, sizes, and colors, such as white, yellow, red, and purple (Mofhammer et al., 2006b). The red prickly pear has an approximate weight of 150.5 g, with dimensions of 5.8 × 5.3 × 9.0 cm.

This fruit can be considered a rich source of a large number of components, such as sugars, vitamin C, pigments, and minerals such as Ca, Na, Mg, Zn, Fe, Mn, and Se (Moßhammer et al., 2006b). Some studies have shown that red prickly pear, when consumed, can protect against oxidative damage (Galati et al., 2003; Gurrieri et al., 2000), has hypocholesterolemic properties, and can inhibit stomach ulceration, in addition to having anti-inflammatory effects (Galati et al., 2003, 2007), hypoglycemic effects and antidiabetic properties. These properties have been related to flavonoid-type polyphenols, which are a very large group of components since more than 6000 flavonoids and derivatives have been identified (Erlund, 2004). Some biological activities are reported for these compounds, including antibacterial, anticancer, antioxidant, antimutagenic, anti-inflammatory, and antiallergic effects (Erlund, 2004; Harborne & Williams, 2000; Hsu & Yen, 2008; Peterson & Dwyer, 1998; Zhang et al., 2010). Among all the flavonoids present in Cactaceae, specifically in prickly pear, flavonol 3-*O*-glycosides (quercetin, kaempferol, and isorhamnetin), dihydroflavonols, flavanones, and flavanonols (Kuti, 2004) has been reported. Although these components have shown the potential to cause beneficial effects on the consumer, they are thermally sensitive, so their thermal processing can cause degradation and therefore change their bioactivity (Heras-Ramírez et al., 2012).

The beneficial effects of the red prickly pear have been related to other components present in the fruit, such as the pigments called betalains, since it has been proven that a large part of the antioxidant activity of the prickly pear is attributable to these pigments (Butera et al., 2002; Castellanos-Santiago & Yahia, 2008; Casterllar et al., 2003), among other effects such as antiviral and antimicrobial activities (Strack et al., 2003). These pigments, betalains, come from betalamic acid, share specific properties, such as being soluble in water, and can be useful to replace synthetic pigments. Betalains are divided into two groups (Fig. 34.2). Betaxanthins have acidic forms and are yellow-orange. The main pigments classified in this group include indicaxanthin, miraxanthin II, and vulgaxanthin I, II, and IV. Betacyanins have basic forms and colors in the red-violet range, and the main pigments classified within this group are betanin, phylocactin, and neobetanin (Stintzing et al., 2001, 2002; Fernández-López & Almela, 2001).

Red prickly pear color is produced by combining the content of betacyanins and betaxanthins, causing a diversity of colors. The color of red prickly pear juice has



**Fig. 34.2** Betalains separation: (a) Betacyanins, (b) Extract of red prickly pear, and (c) Betaxanthins

**Table 34.1** Chemical characteristics of red prickly pear

	Juice <sup>a</sup>	Pulp <sup>b</sup>	Peel <sup>c</sup>	Ethanol extract <sup>d</sup>
Antioxidant Activity	6.36 ± 0.13 (mmol TE/100 g)	26.3 ± 1.8 (μmol TE/g)	262.3 ± 7.70 (mmol TE/kg)	2.87 (mmol TEAC/g)
Total phenolics	14.6 ± 0.14 (mg GAE/g)	–	11.2 ± 0.07 (g GAE/kg)	777.1 ± 4.04 (mg GAE/l)
Quercetin	2.76 ± 0.01 (mg/100 g)	43.2 (μg/g)	10.7 ± 1.43 (mg/kg)	–
Kaempferol	1.62 ± 0.08 (mg/100 g)	2.2 (μg/g)	ND	–
Isorhamnetin	5.22 ± 0.01 (mg/100 g)	24.1 (μg/g)	95.9 ± 0.26 (mg/kg)	–
Betacyanins	1.71 ± 0.01 (mg/g)	–	241.4 ± 1.21 (mg/kg)	0.22 (BE/100 g)
Betaxanthins	0.68 ± 0.01 (mg/g)	–	179.8 ± 0.62 (mg/kg)	10.2 ± 0.008 (mg/kg)

<sup>a</sup>Ruiz-Gutiérrez et al. (2014)

<sup>b</sup>Kuti (2004)

<sup>c</sup>Jiménez-Aguilar et al. (2015)

<sup>d</sup>Sáenz et al. (2009)

values of color parameters for luminosity, greenness-redness tendency, and blueness-yellowness tendency of 43.27, 38.18, and  $-5.51$ , respectively, indicating a dark juice with a major tendency to red and green color (Fig. 34.2b). A separation of the pigment groups is possible; when betacyanins and betaxanthins are separated, each group's specific color can be visualized in the extracts collected. Figure 34.2a shows that the betacyanin group has a light magenta color, and Fig. 34.2b shows that the betaxanthin group has a light yellow color.

An essential factor in a relationship between the contents of the two groups of betalains, which is presented as the ratio between the absorbances of betacyanins and betaxanthins [ $A(536\text{ nm})/A(481\text{ nm})$ ]. This factor is important since it is related to the quality of color, and the optimal range is 1.5–1.8 for color tones (Stintzing & Carle, 2007). Therefore, red prickly pear juice can be considered of suitable quality for use as a food coloring, and a value of 1.75 was reported for red prickly pear (Ruiz-Gutiérrez et al., 2014). Due to the importance of the content of polyphenols, betacyanins, and betaxanthins, as well as the antioxidant activity, Table 34.1 shows the chemical characteristics reported for different fruit parts.

## 4 Extruded Products Formulated Using Prickly Pear

The use of prickly pear in the formulation to obtain extruded products has been tested. Orange-yellow or red pulp was used as concentrates (40°Brix) in formulations to develop new rice-based extrudate products. The addition consisted of mixtures of prickly pear pulp and rice flour at the following ratios: 95:5, 90:10, 85:15,

and 80:20, and 100% rice flour was extruded as a control. The mixtures were extruded at different temperatures using a single-screw extruder, and the results showed that 5% and 10% levels of concentrated cactus pulp (orange or red prickly pear) were the best to produce rice-based extrudates with good functional, nutritional, and sensory characteristics (El-Samahy et al., 2007).

Another extruded product obtained by combining rice and prickly pear was produced by adding orange prickly pear paste and rice flour in three different solid ratios (rice flour solids' ratios: puree solids were 6:1, 8:1, and 10:1). These feed mixes were extruded in a twin-screw extruder using a barrel temperature profile of 25–140 °C at a screw speed of 400 rpm, an L/D ratio of 40:1, and a feed moisture content of 13% (w/w). The cactus pear extrudates' apparent density and breaking strength increased with increasing fruit solid levels. However, the true density, porosity, and radial expansion ratio decreased with increasing fruit solid level. The results of this study indicated that peeled prickly pear could be effectively utilized as a food ingredient for the production of expanded extruded food products and increase the overall fruit utilization (Sarkar et al., 2011).

A different product is an extruded cereal obtained using maize grits (No. 20) and red prickly pear powder, reported by Ruiz-Gutiérrez et al. (2017). In this study, mixtures of maize grits were prepared with 2.5%, 5.0%, and 7.5% (w/w) red prickly pear powder, and maize grits without prickly pear powder were used as a control. The mixtures were extruded using a twin-screw corotating extruder with a 600-mm length, L/D = 20, and a 4 mm die diameter, and the screw configuration was selected specifically to create high levels of shear (Cortés-Ceballos et al., 2015). The mixture was processed at a fixed amount of feed water (0.22 kg water/kg dry matter), a barrel temperature of 100 °C, and a screw speed of 325 rpm. The results showed how the addition of red prickly pear powder to the formulation affected the extruded products' physicochemical characteristics, enhancing properties such as color, and antioxidant activity. Accordingly, red prickly pear powder can serve as a natural alternative to synthetic dyes and can develop functional food with possible health benefits.

## 5 Extrusion-Cooking Effect on the Chemical Properties of Prickly Pear

Incorporating fruits and vegetables into extruded products provides an alternative for consumers interested in making healthier choices. Ingredient mixtures high in bioactive compounds are being studied due to the importance of obtaining products high in antioxidants (Camire et al., 2007; Yağci & Göğus, 2008; Larrea et al., 2005). However, a disadvantage to processing food that contain components such as phenolics and pigments is that the structure of these compounds is affected by the processing conditions, which will also cause changes in their bioavailability and bioactivity. However, retaining these bioactive components is important. Processing

conditions that physically and chemically affect these compounds include pH, water activity, the presence of oxygen and light, and processing temperature. This last factor is one of the most important, as the stability of the components' bioactivity depends on the magnitude and duration of the heating (Sapers & Hornstein, 1979; Casterllar et al., 2003).

Extrusion cooking is a technology based on high temperatures and short times, so the temperature and processing time are closely related to residence times. Knowledge of the residence time distribution (RTD) is important for understanding nutrient degradation, food safety, and product quality (Ilo & Berghofer, 1999); therefore, the material fed to the extruder must be processed within a specific and controllable optimal time. The RTD, which develops as a result of flow and mixing patterns, is highly dependent on process variables, such as the feed material moisture, process temperature, screw speed, and screw and die configuration (Kumar et al., 2008). Several reports present models and predictions of the RTD (Kumar et al., 2008; Braun et al., 2007; Pansawat et al., 2008). Some of the expressions developed have been based on conceptual models of chemical reactors, where simple and multistage models are described, which have served to develop complete RTD models (Kumar et al., 2008) that consider a range of variables that affect the extrusion process. The RTD and mean residence times in the extruder are fundamental parameters since they allow us to explain how the conditions of the extrusion-cooking process affect the degradation and stability of the bioactive components of interest as well as their conversion to other components, directly affecting the composition, functionality, and acceptability of the final product. Likewise, the RTD provides useful information in scaling up and moving processes. This knowledge also makes it possible to facilitate and improve the design and manufacture of extruded food that are high in fiber or antioxidants so that their consumption results in health benefits, thus reducing cardiovascular and chronic diseases.

A common way to determine the RTD and mean residence time ( $tr_m$ ) is using the procedure proposed by Levenspiel (1972); calculations are presented in Fig. 34.3.

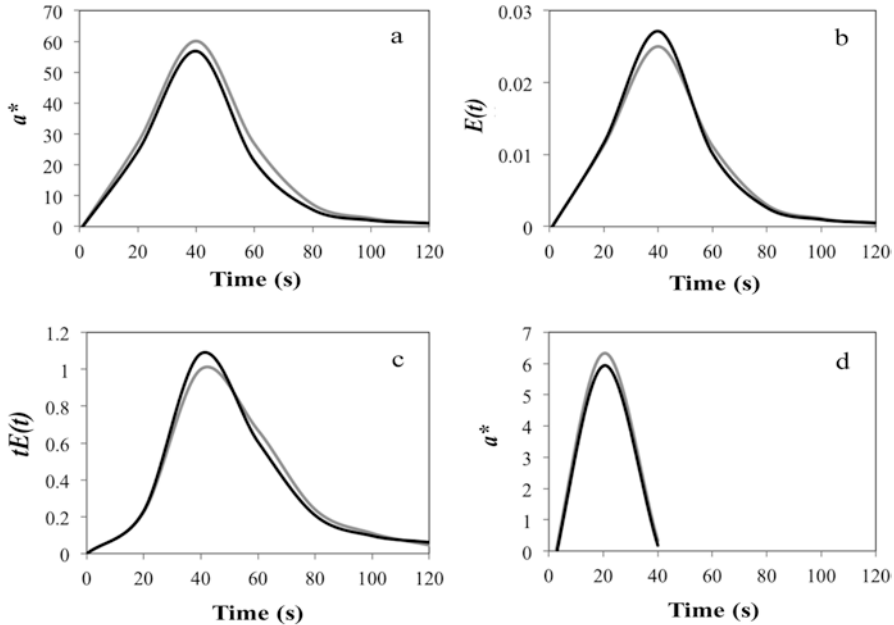
First, a concentration-time curve is constructed, given in the following equation:

$$\int_{\infty}^0 C(t) dt \quad (34.1)$$

The curve  $C$  obtained is useful for the construction of the residence time distribution function  $E(t)$ , which quantitatively describes how long different elements have spent in the extruder and is given in the following equation:

$$E(t) = \frac{C(t)}{\int_0^{\infty} C(t) dt} \quad (34.2)$$

The calculation of  $tr_m$  is given by Eq. (34.3) and represents the average value of the time that the material was in the extruder.



**Fig. 34.3** Distribution of material residence times in a twin-screw extruder (325 rpm and 100 °C); (a) Concentration-time graph, (b) Residence time distribution function, (c) Average residence time graph, and (d) Standard deviation

$$tr_m = \int_{-\infty}^0 tE(t) dt \quad (34.3)$$

where  $i$  is the sample index,  $t$  is time, and  $E(t)$  is the residence time distribution curve.

The  $tr_m$  is affected mainly by the barrel temperature and the screw speed, and increases in the screw speed in the extruder at the same temperature cause a decrease in the mean residence time of the material (Ruiz-Gutiérrez et al., 2015; Pansawat et al., 2008; Plunkett & Ainsworth, 2007). This is probably due to greater heat dissipation, greater shear stress, and better mixing of the material (Unlu & Faller, 2002; Ilo & Berghofer, 1999). When the barrel temperature increases, a decrease in  $tr_m$  is observed, probably due to a decrease in the viscosity of the material in transit, which produces a more fluid material through the barrel and the screw (Unlu & Faller, 2002; Ilo & Berghofer, 1999). The  $tr_m$  at 80–140 °C temperature and 225–325 rpm screw speed ranged from 36.62 to 60.13 s using a twin-screw corotating extruder (BCTM-30, Bühler, Uzwil, Switzerland) with a 600 mm length, and L/D = 20 (Ruiz-Gutiérrez et al., 2015).

The calculated  $tr_m$  can be used to model the degradation of pigments processed by extrusion cooking. Reports show that the degradation of betalains follows first-order kinetics (Chandran et al., 2012), and accordingly, the loss of betalains can be described by the following equation:

$$\ln(C_t - C_0) = -kt \quad (34.4)$$

where  $C_0$  is the initial measurement of the component,  $C_t$  is the measurement at time  $t$ ,  $k$  is the reaction rate constant, and  $t$  is time. The dependence of the reaction rate constant ( $k$ ) on temperature can be described by the Arrhenius equation:

$$k = A_0 \exp(-E_a / RT) \quad (34.5)$$

where  $A_0$  is the frequency factor (1/s) as a pre-exponential constant,  $E_a$  (J/mol) is the activation energy of the reaction,  $R$  (8.3143 J/K mol) is the universal gas constant, and  $T$  is the absolute temperature (K).

Knowledge of the kinetic parameters allows us to evaluate the effect that some process variables exert on the stability of the pigments and, therefore, the stability of the properties that they present and makes it possible to obtain optimized conditions for the process to obtain products with a high content of components of interest.

Red prickly pear has been added to the formulation of extruded products to take advantage of the functional properties or the potential pigmentation, and the form used has been pulp (El-Samahy et al., 2007; Sarkar et al., 2011) or powder (Ruiz-Gutiérrez et al., 2015, 2017). The application as a powder is because the high sugar and moisture in most fruits limit their use, and spray-dried powders and concentrates can be easily incorporated into product formulations. In general, the addition of prickly pear increases nutraceutical properties (El-Samahy et al., 2007), but phenolics, betacyanins, and betaxanthins are affected by the extrusion-cooking process, modifying their antioxidant activity and color.

Many studies monitor the changes in phenolics, such as anthocyanins, flavanols, and phenolic acids caused by extrusion cooking. Specific studies using red prickly pear in the formulation of extrudates have shown that the extrusion-cooking process reduces the total phenolics present in raw mixtures. The phenolic content is reduced by increasing the temperature, but when the screw speed is increased, the content is less affected, attributed to the decrease in the residence time obtained by increasing the screw speed. The total phenolic content is reduced during the processing, and the retention values calculated for the different extrusion conditions ranged from 36% to 47% at temperatures higher than 80 °C and high screw speed (>225 rpm) in a twin-screw extruder. Temperatures over 80 °C may destroy or alter polyphenols' nature (Zieliński et al., 2001). Some explanations of the reduction of phenolics are as follows:

1. Decomposition or alterations in the molecular structure of phenolic compounds are caused by the high extrusion temperature (Altan et al., 2008).
2. Free phenolic acids undergo decarboxylation during the extrusion process (Dlamini et al., 2007).
3. Insoluble complexes form with food components such as proteins (Dlamini et al., 2007)

4. High temperature and feed high moisture content can promote the polymerization of phenolics, reducing their extraction capacity and chemical reactivity, thereby making quantification difficult (Repo-Carrasco-Valencia et al., 2009; Ti et al., 2015; Brennan et al., 2011).

Although other reports, by optimizing the processing conditions, have found that the process increases phenolic compounds' content and improves the antioxidant capacity, specifically sorghum bran was used (Ortiz-Cruz et al., 2020). Flavonoids are compounds found in food, as prickly pear, in low content ( $\mu\text{g/g}$ ); that is why monitoring them during the process such as extrusion-cooking is difficult.

The contents of the color compounds, betacyanins, and betaxanthins, are reduced by the extrusion-cooking process. Betaxanthins are yellow-orange pigments that are affected by temperature action and by changes in screw speed. A longer residence time of the mixture in the extrusion barrel represents a longer exposure time of the thermosensitive components to process conditions such as high temperature, which increases the degradation of the pigments since they are sensitive to heat (Fernández-López & Almela, 2001). The retention percentages reported for betaxanthins are between 46% and 63.5%, concerning the mixture fed to the twin-screw extruder at temperatures higher than 80 °C and high screw speed ( $>225$  rpm), showing the high sensitivity of these pigments. The main betaxanthin present in red prickly pear is indicaxanthin, and studies have demonstrated that isomerization of indicaxanthin will be induced by thermal exposure (Moßhammer et al., 2006a). If both pigment groups' stability is compared, betaxanthins are more susceptible to temperature increases (Wei-Dong & Shi-Ying, 2007). During the extrusion-cooking process, the activation energy for the degradation of betaxanthins was 3.9 times the activation energy for the degradation of betacyanins (Ruiz-Gutiérrez et al., 2015).

On the other hand, for betacyanins, red-purple pigments, an increase in temperature, together with an increase in screw speed, can lead to greater retention of these pigments. This can be explained through the average residence time. When the temperature and the screw speed in the extruder are increased, the residence time of the material inside the extruder is reduced, possibly due to the decrease in shear stress resulting from the decrease in the viscosity of the melt at high temperatures (Björck & Asp, 1983). The calculated retention values were in the range of 33% to 51% (Ruiz-Gutiérrez et al., 2015). It has been reported that betanin is the main pigment of red prickly pear and can be degraded by isomerization or decarboxylation during heat treatment (Huang & von Elbe, 1985). Pigment loss caused by the extrusion-cooking process has been previously reported. Durge et al. (2013) reported retention of 50% for beet pigments processed by extrusion cooking; however, only the color retention was measured in this study, not the presence of the compounds.

The betacyanins are compounds responsible for the red-purple color in red prickly pear, and the main betacyanins are betanin and isobetanin. After the extrusion-cooking process, the betanin retention, with applied temperatures between 80 and 140 °C at high screw speeds ( $>225$  rpm), ranged between 10.9% and 43.5%,



with higher retention at lower temperatures and lower screw speeds. In contrast, isobetanin showed a trend opposite to that observed for betanin, and an increase in the isobetanin content was observed under all extrusion conditions. There are structural changes related to the effects of temperature on betanin and isobetanin:

1. The epimerization of betanin to isobetanin may be caused by heat (Fennema, 2000).
2. The high temperature applied in extrusion can lead to the isomerization and decarboxylation of betanin, producing its C15 stereoisomer, corresponding to isobetanin (Stintzing et al., 2006).

The reductions in the compounds that produce color are reflected in color changes; when compounds such as phenolics, betacyanins, and betaxanthins are degraded or modified, color is also affected. The following describes how the parameters of color, lightness ( $L$ ), a tendency from green to red ( $a$ ), and tendency from blue to yellow ( $b$ ) are affected by the main variables of the extrusion-cooking process, such as the temperature and screw speed, as described by Ruiz-Gutiérrez et al. (2015).

A high extrusion temperature (140 °C) and a high screw speed (325 rpm) can cause the luminosity to decrease compared to that obtained at lower speeds and temperatures, which can indicate the formation of some products of Maillard reactions that cause darkening in the extrudates (Sacchetti et al., 2004). Despite the effect on luminosity, using high screw speeds even when the temperature is high, greater red color retention is observed. This trend may be due to greater conservation of the components that provide red color, such as betacyanins, caused by the shorter residence time of the material in the extruder that will be achieved with increasing temperature and speed. However, it may also be due to the increase in the formation of products of the Maillard reaction due to the increase in the temperature of the process (MacDougall & Granov, 1998). Something similar happens for the color parameter  $b$ : when the barrel temperature in processing is increased, the tendency to yellow increases and the highest values of  $b$  were found in the extrudates obtained at the highest processing temperature (140 °C). The situation may be the same as when explaining  $L$  and  $a$ : the use of high temperatures can cause increase the formation of products of the Maillard reaction (MacDougall & Granov, 1998).

The relationship between the variables has been evaluated. The correlation between compound content and color has been calculated. The  $L$  parameter (luminosity) presented a positive correlation (0.991) with the  $b$  parameter and negative correlations concerning the  $a$  parameter (−0.993), phenolic content (−0.961), anti-oxidant activity (−0.975), betacyanin content (−0.954), and betaxanthin content [the  $a$  parameter (tendency to red color) presented a positive correlation (0.921) with the betacyanin content (0.934)]. The  $b$  parameter was negatively correlated with betalains, betacyanins (−0.921), and betaxanthins (−0.936).

## 6 Effect of the Addition of Prickly Pear on the Physical Properties of Extruded Products

The use of prickly pear in the formulation of extruded products is an alternative for obtaining functional products or products with a clean label since it is possible to replace synthetic colorants with natural pigments. However, obtaining functional products or clean label products is a challenge because changing the traditional, inexpensive, or stable formulation changes the physical and sensorial properties. The extruded product characteristics of breakfast cereal, filled products such as energy bars and pasta determine whether the consumer will select the product. Therefore, the effect of adding or changing an ingredient in the formulation must be evaluated to determine the impact of the ingredient on both the processing and the quality of the extruded product obtained.

The addition of prickly pear pulp from orange-yellow fruit and red fruit and its effects on extrudates' main physical properties have been studied, and specific values are summarized in Table 34.2. Different additions of pulp fruit have been tested (5–20%), and changes in physical properties were reported. As the pulp content increased, the expansion ratio, water-soluble index, and water absorption index of the extrudates decreased, while the breaking strength showed no trend and the bulk density, ash content and color attributes increased (El-Samahy et al., 2007). Other studies have also described how increased levels of orange fruit solids in the mixtures decreased the true density, porosity, radial expansion ratio, axial expansion ratio, and an overall expansion ratio of the extrudates. The apparent density and breaking strength showed an opposite trend with increasing fruit pulp (Sarkar et al., 2011). Another form in which prickly pear can be added is a spray-dried powder, at levels of 2.5–7.5%. As the percentage of powder in the extrusion material increased, there was an increase in the bulk density, expansion index, and water absorption index. However, the water solubility index was not affected.

The changes in the properties can be explained as follows:

- The bulk density is affected by sugars, fibers, and other compounds such as pigments that replace the starch content (Andersson et al., 1981), preventing expansion and increasing the density. The presence of compounds such as phenolics or compounds with hydroxyl groups (betalains) that can form complexes with starch (Bordenave et al., 2014) compacts the matrix to increase the efficiency of molecular packing (Vargas-Solórzano et al., 2014).
- The expansion index is decreased due to the dilution effect caused by the powder in the starch (Altan et al., 2008; Khanal et al., 2009). Moreover, when polysaccharides other than starch are present in the mix, they can hydrate and compete with the starch for the water at the extruder outlet, thereby restricting the starch gelatinization process (Khanal et al., 2009; Yanniotis et al., 2007).
- The change in the water solubility index has been attributed to diluting the starch content in the cereal. Another explanation is that the soluble fiber present in the powder negatively affects the gelatinization and dextrinization of the starch,

**Table 34.2** Physical properties evaluated in extruded products formulated with prickly pear

Ingredient	Property			
	Expansion	Water solubility index	Water absorption index	Bulk density
Orange Pulp <sup>a</sup>	2.72–3.22	29.7–33.5%	8.63–9.83	78.7–88.3 (g/100 cc)
Orange Pulp <sup>b</sup>	6.66–12.3	–	–	116.0–229.6 (kg/m <sup>3</sup> )
Red Pulp <sup>a</sup>	2.76–3.15	29.3–33.7%	8.88–9.19	82.7–87.9 (g/100 cc)
Red powder <sup>c</sup>	4.66–9.43	0.23–0.24	4.81–5.87	0.106–0.309 (g/cm <sup>3</sup> )

<sup>a</sup>El-Samahy et al. (2007)<sup>b</sup>Sarkar et al. (2011)<sup>c</sup>Ruiz-Gutiérrez et al. (2017)

leading to extruded products with a lower capacity to absorb water (Altan et al., 2008). Another possible explanation is that compounds (phenolics and pigments) in the powder may interact to form noncovalent interactions with amylose, leading to changes in the starch's textural properties, which then affect starch gelatinization and retrogradation (Bordenave et al., 2014; Barros et al., 2012).

One of the objectives of using pulp or spray-dried powder of colored prickly pear fruit is to replace the synthetic colorants in extruded products with natural pigments for multiple reasons. First, some health problems have been associated with the use of synthetic colorants, especially in children; second, the natural pigments give consumers an alternative to functional products; and third, natural colorants continue the trend of manufacturing clean label products, which have become popular for their lack of harmful effects on health.

Another important physical property of extruded foods that is affected by the addition of prickly pear is the color parameters. The color parameters are  $L$ ,  $a$ , and  $b$ , luminosity (0–100), greenness (–) to redness (+) tendency, and blueness (–) to yellowness (+) tendency, respectively. The extrudates' color depends on the raw material added to formulate the mix, and the color parameters obtained in some products are presented in Table 34.3. In general, the  $L$  parameter is reduced by the addition of prickly pear, which means the tendency to white is decreased. The same behavior is observed regardless of the red prickly pear's color and the way it is added (pulp or powder).

Parameters  $a$  and  $b$  are affected because the extrudate's color depends on the color of the fruit that is being used. When orange or red prickly pear pulp is used, the parameter  $a$  changes slightly as the amount of pulp added to the mixture increases. It remains at low levels but tends toward red. The  $b$  parameter is similar for both red and orange pulp, and although increases in the pulp increase it, the change is slight. When red powder from red prickly pear was tested, both the  $a$  and  $b$  parameters were higher than those obtained using pulp, but the behavior was the opposite. The tendency to red color ( $a$  parameter) increased as the powder was increased, but the tendency to yellow color ( $b$  parameter) decreased with increasing powder content. The explanation is that the structures of compounds with yellow color, such as phenolics and betaxanthins, were modified or degraded by the process enough to reduce the yellow color (Ruiz-Gutiérrez et al., 2017).

**Table 34.3** Color parameters of extrudates using prickly pear in the formulation

Parameter	Ingredient		
	Orange pulp <sup>a</sup>	Red pulp <sup>a</sup>	Red powder <sup>b</sup>
L	76.3–79.2	74.9–82.8	61.3–76.6
a	7.0–8.1	4.9–8.8	15.3–32.2
b	18.7–23.6	19.8–24.0	21.9–52.8

<sup>a</sup>El-Samahy et al. (2007)

<sup>b</sup>Ruiz-Gutiérrez et al. (2017)

## 7 Sensory Properties of Extruded Products Formulated Using Prickly Pear

Any product produced by extrusion cooking or another processing method must be evaluated for its characteristics or functional properties. The addition of orange-yellow or red prickly pear pulp to rice-based extruded product, using mixes of 95:5, 90:10, 85:15 and 80:20 rice: pulp, resulted in a clear improvement in crispness, which was significantly improved by increasing the substitution level of the prickly pear concentrates to 10% from the base formula (0% prickly pulp). This agrees with the results obtained above for breaking strength values, which were decreased by adding 5% and 10% prickly pear concentrates and then increased at the highest levels of substitution. Extrudates manufactured with 5% and 10% orange prickly pear pulp obtained the highest scores in taste, odor, crispness, chewiness, color, and overall acceptability, with this last rated as “Good”. For red prickly pear pulp use, the addition of 5%, 10%, or 15% resulted in higher scores for odor, crispness, and chewiness, but 15% had relatively low scores for taste and color, whereas concerning overall acceptability, a 10% addition was qualified as “good”; the above evaluation was performed by trained panelists (El-Samahy et al., 2007).

On the other hand, extruded cereal produced with different percentages of red cactus pear powder showed how consumer judges’ preferences classified texture, color, taste, and odor attributes. The results obtained from the sensory analysis showed that cereal extrudates could be divided into two groups: low content (0% and 2.5%) and high content (5.0% and 7.5%) of powder. The highest powder content’s cereals were the hardest and were classified as neither liked nor disliked by judges. The lowest powder content’s cereals had the lowest hardness and were classified as moderately liked by the consumers. Concerning color, the most liked extruded cereals had a red color (7.5% powder), and the least liked had a pink color (2.5% and 5% encapsulated powder or control cereals). However, for taste, the consumers preferred cereal without added powder and those with 2.5% powder, and the lowest appeal was for cereal with a high content of red prickly pear (7.5%). Regarding odor analysis, the consumers reported a corn smell from the extruded cereal samples (Ruiz-Gutiérrez et al., 2017).

Breakfast cereal or extruded cereal is a ready-to-eat product, but it is not consumed alone; the cereal is consumed in a bowl with milk, which changes the choices or scores designated by consumer judges. According to judges’ comments, the texture for cereal with the lowest content of red prickly pear powder (0%-control, and 2.5%) was much softer when soaked in milk, whereas the cereals with higher contents of red prickly pear encapsulated powder did not soften easily. The addition of 5.0% and 7.5% encapsulated powder did not achieve sufficient sensory acceptance due to the elevated hardness cereals, but cereal with 2.5% red prickly pear powder was preferred over nonpigmented cereal.

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# Chapter 35

## *Opuntia* spp. and Extruded Food



**Muhammad Imran, Muhammad Nadeem, Muhammad Kamran Khan, Muhammad Haseeb Ahmad, Rabia Shabir Ahmad, Aurbab Shoukat, and Muhammad Abdul Rahim**

**Abstract** *Opuntia* spp., the prickly pear fruit, also known as cactus pear, belongs to the cactus family, Cactaceae. The fruits are the well-known raw material that is considered a significant source of bioactive substances (anthocyanin, betalains, carotenoids, flavonoids, and phenolic acids), vitamins, and minerals with health-promoting properties. Therefore, the potential composition of *Opuntia* spp. fruits is increasingly gaining momentum for functional, nutritional, technological, and medicinal applications. Furthermore, *Opuntia* spp. fruits have increased food industrial interest due to proper mixing and blending properties during thermal processing. As a relatively modern HTST processing technology, extrusion cooking is extensively used to develop new food products supplemented with functional ingredients. However, the functional and technological applications of whole *Opuntia* spp. fruit in extruded food products is limited in the food processing industry. The data related to the supplementation of *Opuntia* spp. fruit in extruded expanded food products is scattered. The present literature summarizes data from promising research areas focusing on expanded food products enriched with components or wholesome *Opuntia* spp. fruit.

**Keywords** Food technology · Cactus pear · Prickly pear · Composition · Functional · Technological · Extrusion

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## 1 Introduction

*Opuntia*, generally known as prickly pear, belong to the family Cactaceae and is widespread in the world's semi-arid and arid regions, traditionally with about 300 species of the *Opuntia* genus. The cactus is an *Opuntia* with unusual shapes and beautiful flowers (Majure et al., 2012). It is known for its taxonomic difficulty due to its no sexual compatibility as it contains more than two sets of chromosomes. It is mainly controlled by environmental and endogenous factors and genetics. *Opuntia* has survived cultivation and has become invasive around the world. It has been commonly used in the world's semi-industrial countries as the United States, Africa, and Australia for thousands of years (Ervin, 2012). It is a flattened organ that grows from a plant's stem and is covered with thorns and tiny hairs. The prickly pear has the long up to 2 in. thorns, and due to the presence of betalains, the nopal may have different colors such as red, yellow, white, purple, and different shades of green (Reyes-Agüero et al., 2005). Cactus pear or *Opuntia ficus-indica* is widely introduced as a commercial fruit and vegetable with no thorns (AL Vigueras & Portillo, 2001). Cactus species are used for a variety of purposes but are most commonly used in food products. Nowadays, people worldwide are more interested cactus derived foods because of their excellent nutritional, nutraceutical, pharmaceutical, and medicinal properties (Gurrieri et al., 2000; Lee et al., 2002; Stintzing et al., 2002; Tesoriere et al., 2005; Ndhilala et al., 2007).

The succulence and nutritive values of the prickly pear cactus are an excellent source of essential amino acids that are important for the body and brain. According to the previous studies, the main components of minerals and vitamins are found in *Opuntia* such as magnesium (Mg), calcium (Ca), iron (Fe), potassium (K), sodium (Na), phosphorus (P), and vitamin C. Its rich composition in nopal is complex carbohydrates, phenolics, and antioxidants. Paddles are a rich source of dietary fiber in which betalains is the most beneficial (El-Mostafa et al., 2014). Hernández-Urbiola et al. (2010, 2011) performed studies on the nutritional composition of nopal in which results showed that the nopal contains dry matter 10%, antioxidant 0.36%, minerals 1.96%, fat 0.72%, and glycan 4.8%, wherein nitrogen contents were not detected. Several studies on the chemical composition of nopal were conducted. The study of Stintzing and Carle (2005), indicated that *Opuntia* contains 6.4–7.1% of carbohydrates, 0.1–4% fat, 1.9–2.3% mineral and protein in the range of 0.4–1%. Furthermore, they also determined the fresh nopal chemical composition and reported 0.3–0.7% carbohydrates, 0.05–0.1% protein contents, 0.02% lipid contents, and 0.1–0.2% ash contents. Galati et al. (2003) conducted research to extract the *Opuntia ficus-indica* juice using the mechanical pressing method. This study's results showed that the juice contained polyphenols in the form of glycosides such as flavonoid-3-*O*-glycosides and their derivatives (Galati et al., 2003). *Opuntia ficus-indica* also contained quercetin in the form of aglycone (Kuti, 2004). Besides, the *Opuntia ficus-indica* contains various pigments, wherein the unique yellow indicaxanthin and purple-red betanin are important pigments (Stintzing et al., 2002). *Opuntia ficus-indica* is used as a folk medicine. It is very effective against many

diseases and conditions, including low-density lipoproteins peroxidation, chronic diseases, rheumatic pain, asthma, and oxidative stress (Galati et al., 2003; Ennouri et al., 2007; Chang et al., 2008; Chavez-Santoscoy et al., 2009).

## 2 Extrusion Processing

Extrusion is a metal forming process or hot forming method used to make the raw material of a fixed cross-sectional profile through a die to convert it to the desired shape (Harper, 1981). In this process, raw material or object is exposed to control conditions such as water contents, pressure, and temperature. It is an advanced technology in the food industry and livestock feed processing. This technology has become more important in commercial food production, aquatic feed, and animal feed as efficient production methods. The process involves high temperature for a short time and faster processing. It is essential food extrusion processes in preparing corn meal snacks, dry pet foods, ready-to-eat food, cereals, fried snacks, and vegetable proteins (Sumathi et al., 2007). Extrusion cooking is used to convert raw material into food ingredients; for this purpose, the extruder is an excellent choice (Choton et al., 2020).

There are two types of extruders in the industry, “single screw extruder and twin-screw extruder”. Twin-screw extruders have limited use due to their relatively high cost of process and maintenance (Hauck, 1990). Some of the advantages of food extrusion are versatility, flexibility in use, and high productivity. Besides, it has other advantages such as relatively inexpensive, different shapes of product, good quality of the product, the end product has excellent nutritional value, require less energy to perform, new product development, long shelf life at room temperature, and zero waste (Lusas & Rooney, 2001). A study conducted by de Gutiérrez and Gómez (1987) indicated that extrusion cooking leads to starch granules to swell, protein denaturation, and endogenous food enzymes inactivated, toxins as a naturally-occurring constituent destroyed by cooking and finished products demonstrate better quality.

## 3 *Opuntia* spp. Extruded Products

Figure 35.1 presents the raw materials, and extruded food products from cactus pear. Red cactus pear juice was extracted using the mechanical extraction method. Furthermore, the juice microcapsules or powder were prepared using the spray drying method, and the drying process was carried out in different variations such as independent variable inlet temperature 160 °C and dependent variable outlet temperature 85 °C. The experimental food product was prepared using the mixture of native cornstarch and encapsulated red cactus pear powder as functional ingredients. For this purpose, a twin-screw extruder was used to make an experimental

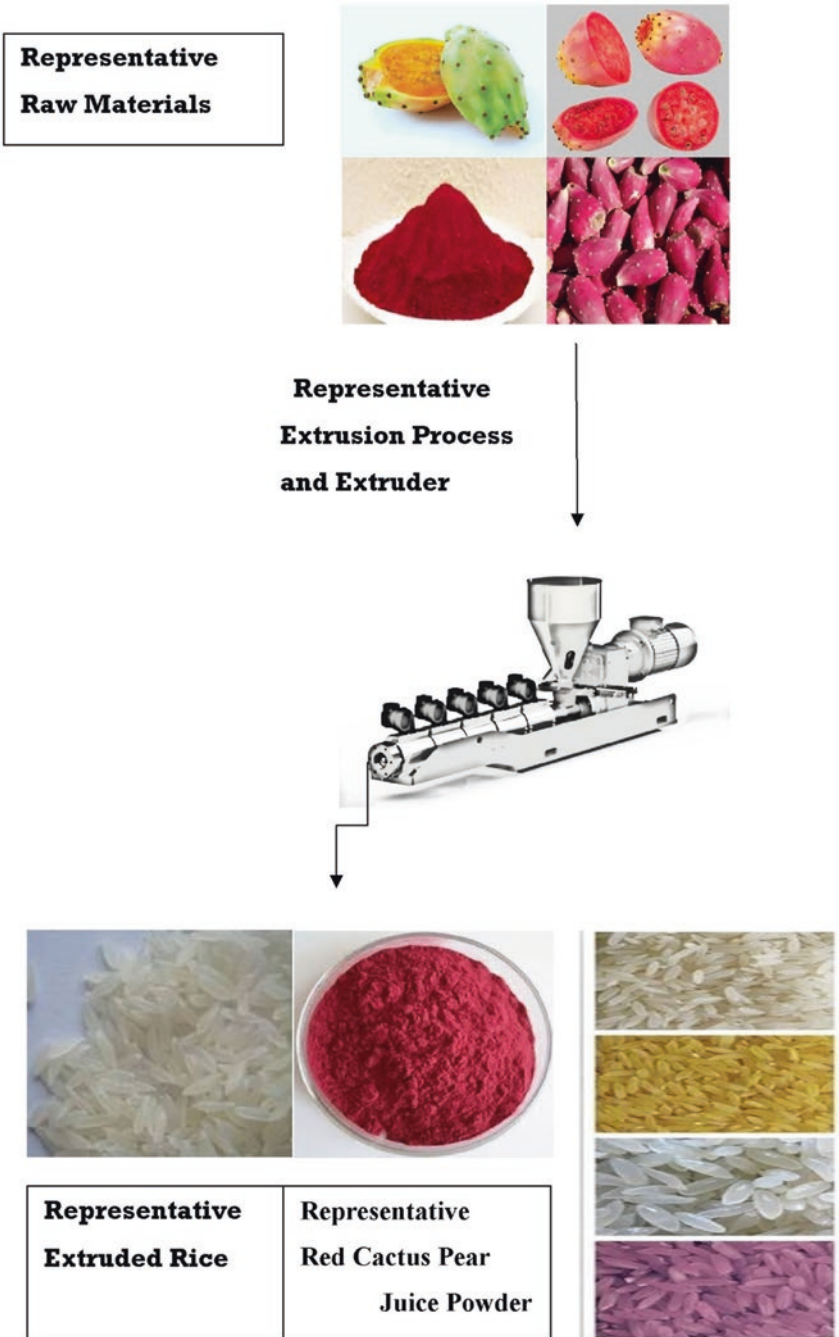


Fig. 35.1 Raw materials, and extruded food products from cactus pear

food product. The mixture was fed into the twin extruder at different barrel temperatures and screw speeds. According to the treatment plan, the processing conditions were set for extrusion, such as a fixed amount of 0.22 kg feed water at different temperatures of 80–140 °C, and screw speed was adjusted to 225, 275, and 325 rpm. The mean residence time values of the material used in the extruder ranged from 36.6 to 60.1 s. The results indicated that the temperature and screw speed of the extruder was directly proportional to mean residence time. Therefore, the barrel temperature and screw speed were increased than the value of mean residence time values. The mean residence time values had a significant effect on the value of chemical characteristics, indicating better retention of antioxidant activity and total phenolics in the product. Effluent samples were taken from the extruder every 20 s. The factors were evaluated for extrudate analyses, and the highest level of moisture content of 9.26% was noted. The highest values of color were obtained at screw speeds of 225 rpm and process temperature (140 °C) 25.2%, similarly the highest value of antioxidant activity ranged from 55% to 77% at the highest screw speed 325 rpm and highest temperature. The results concluded that the extrusion cooking process affected the retention of powder, and to maximize the antioxidant properties of the product, red cactus pear juice can also be used as color pigments in food industries (Ruiz-Gutiérrez et al., 2015).

Another study was conducted by Ruiz-Gutiérrez et al. (2017), wherein red cactus pear powder was prepared using a spray drying method, encapsulated powder, and maize grits were mixed and extruded at 100 °C, and the screw speed was 325 rpm. The physical analysis of all cereal extrudates differed significantly and expansion. The color pigment in the encapsulated powder improved, but the density and texture value was reduced. The results obtained from this sensory analysis were overall acceptable.

The experimental food product was prepared using rice flour with *Opuntia* pulp in three different ratios (10:1, 8:1, 6:1). *Opuntia* pulp and rice flour solids were mixed, and the moisture in all mixtures was adjusted to 13%. There was a decrease in the overall expansion of the extrudates. Therefore, the increase in abundance while reducing the actual density with breaking strength indicated an anti-phenomenon with increasing surface area of *Opuntia* pulp. The results confirm that the feed mixture's high sugar content has reduced the expansion of extrudates (Setia, 2010).

Half-ripe orange-yellow and red cactus pear fruit extracted pulps with rice flour were used as functional ingredients to prepare a delectable product. Different pulp ratios extracted with a mixture of rice flour were used in extrusion cooking (95:5, 90:10, 85:15, and 80:20). In this study, a single-screw extruder was used to process the different formulations at the optimum processing conditions. The result indicated that extruded product's functional characteristics were reduced by enhancing the concentration of extracted pulp. Furthermore, no significant difference was shown between the extrudates' chemical composition; the sensory properties of 10% concentration of cactus pear were improved compared to other treatments. The final results concluded that 5% and 10% concentrated cactus pulps are best for producing extruded products (El-Samahy et al., 2007).

Research was carried out by Rayan et al. (2018) to know the effect of cactus *Opuntia* on ice-based extrudates' chemical composition. The seeds powder of *Opuntia* and the rice grits were mixed in eight different ratios 0%, 2%, 4%, 6%, 8%, 10%, 15%, and 20%. The mixture was placed at room temperature until the moisture content of the mixture was 16%. The single screw extruder was used to process the prepared formulas, and the feeding temperature was adjusted to 100 and 180 °C in which the screw speed was fixed between 250 and 160 rpm. The expansion of the extrudates was reduced by increasing the powder's concentration, and the breaking strength of the samples was lower with the lower concentration of the powder. Furthermore, the seed powders' addition increased the oil absorption index compared to the control sample. Sensory analysis of the samples was improved by additional seed powder.

Sarkar et al. (2011) conducted research using prickly pear fruit solids in extruded food products. Prickly pear fruit was blended to make a paste. This paste was mixed with different ratios of rice flour. For this purpose, the mixture was fed into the twin-screw extruder at optimum processing conditions. The experimental products were analyzed for physical and texture characteristics. The porosity ranged from 86.1% to 70.3%, and for true density ranged from 837.8 to 775.8 kg/m<sup>3</sup>. The physical analysis of extruded products increased by increasing the added concentrated fruit ratio.

*Opuntia ficus-indica* fruits' peels contained more flavanols, phenolic content, and natural antioxidant than corresponding pulps. *Opuntia ficus-indica* fruits' peels and pulp were mixed, using a freeze-drying method to dry at low temperature. The different ratio of freeze-dried samples was used to prepare experimental food product with rice powder and corn grits. HPLC-DAD was used to detect the flavonols in the sample. A single screw extruder was used to prepare the fortified snacks at optimum conditions. The results showed that the flavanol content in the freeze-dried sample was 148 µg/100 mg, and ultimately the antioxidant activity of the fortified snacks was improved (Moussa-Ayoub et al., 2015).

## 4 Future Recommendations

1. Further research is needed to find out the effect of *Opuntia* and in combination with cereal on the physical and textural properties of the extruded products.
2. Peeled pears can be effectively used as a nutrient to prepare expanded extruded food products and increase overall fruit consumption.
3. The prickly pear fruit peel powder contains ascorbic acid, minerals, and color attributes, including sugar, proteins, and vitamins that make this powder interesting for human food.
4. Cactus pear fruits can be used to develop various food products as it is a potential source of micronutrients.
5. The unique flavanol profile of *Opuntia* peel can act as a biochemical marker to test the authenticity of the products.

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# Chapter 36

## *Opuntia* spp. Extruded Food Products



Sibel Yağci

**Abstract** Extrusion is an ideal processing method for manufacturing a wide range of food with a long shelf life. Although cereals occupy a large portion of the extruded food market, several other types of raw materials can be used in the formulations in which the fruits become popular due to their functional properties. Nowadays, the production of healthier extruded products is huge, as consumers increasingly focus on consuming nutritious and convenient snacks enriched with functional ingredients like dietary fiber, mineral, and antioxidant. *Opuntia* spp., often called “prickly pear” or “cactus pear”, is an emergent crop that evolved commercially during the second half of twentieth century. Recently, *Opuntia* cactus has been recognized as a functional raw material and source of nutraceuticals because of high fiber (mainly pectin) and mineral (iron, calcium, potassium, and magnesium) contents and also antioxidant properties related to ascorbic acid, flavonoids, betalains, carotenoids, and  $\beta$ -carotene. Up to now, the information on the usage of cactus pear in the extrusion process is still scarce. A few research studies have been conducted on extrusion processing of cactus pear, although it has desirable characteristics like attractive color, high sugar content, and rich nutrients, making it a perfect ingredient for extrusion. Information presented in this chapter would be necessary for processors and researchers to enhance extruded products’ nutritional quality by incorporating *Opuntia* spp.

**Keywords** Cactus-cereal snack · Extrusion · Cactus pear · Health-promoting effect

### Abbreviations

DF	Dietary fiber
DHA	Decosahexaenoic
DPPH $\cdot$	1,1-Diphenyl-2-picrylhydrazyl radical
EPA	Eicosapentaenoic

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HPLC-DAD	High performance liquid chromatography with diode-array detector
HTST	High temperature short time
IDF	Insoluble dietary fiber
L/D	Length to diameter
RS	Resistant starch
SDF	Soluble dietary fiber
TDF	Total dietary fiber

## 1 Extrusion Technology in Food Processing

Extrusion is a widely used process and can be defined as the operation of shaping a dough-like material by forcing it through the restricted die to form the desired shape and structure. Raw materials used in the extrudate production are fed to the barrel in which the mass is conveyed to the die with one or two rotating screws. The equipment used in this process is called an extruder (Yağcı et al., 2014). The food extruder must complete several operations in a short time under controlled, continuous, and steady-state conditions. The combination of operations is possible because of many controllable variables such as feed rate, total moisture of mass, screw speed, barrel temperature, screw profile, and die configuration (Thymi et al., 2005). Raw materials are mixed, and heated/warmed up, while they are conveyed forward in the extruder's barrel. Shear forces applied in mixing are mostly due to the screw speed and the screw/screws structure (i.e., screw geometry). Shear forces increase with increasing screw speed. Shear forces can also be increased if there are mixing elements (e.g., mixing blades), areas that stop material (e.g., reverse screw elements), or areas in which volume between the screw and the surface of the barrel is decreasing in screw (Yağcı et al., 2014). According to the extrusion conditions used, and products obtained, extrusion processes of food products are usually classified into three categories (Rokey, 2000):

- **Low-shear extrusion (cold forming).** The density of the material is usually high in this case. Both the barrel and the screw speed temperatures are held low, resulting in high moisture content in the final product (e.g., pasta). Mixing and forming occur at temperatures lower than 100 °C.
- **Medium-shear extrusion.** This is used to process raw materials with lower moisture content with higher energy inputs (e.g., pet foods, aquatic feeds, texturized vegetable protein).
- **High-shear extrusion (high-pressure extrusion).** Screw speed and energy inputs through the extruder barrel (usually have low L/D ratio) are very high in order to achieve temperatures higher than 100 °C. Highly expanded products are obtained (e.g., snacks, breakfast cereals).

## 2 Advantages of Extrusion Cooking

Extrusion cooking technology offers several advantages over traditional methods of food processing. Many extruded products with different shapes, textures, colors, and appearances can be produced using extrusion as an energy-efficient processing. An extruder provides continuous high-throughput processing and can be fully automated. Extrusion processes are also easy to scale-up from pilot plants to extensive production. The extrusion also produces little or no waste streams (Riaz, 2001; Guy, 2001).

One crucial advantage of the extrusion process is producing extrudates with different forms and products with puffed, and crispy structure. In the high-shear extrusion process, moistened, starchy, and/or proteinaceous materials are compressed, sheared, and heated to form a melt. Because the exit temperature can reach as high as  $\sim 150$  °C, some cooking of the starches occurs during the extrusion process in addition to the shearing (Burtea, 2001). When a pressurized melt goes through the die, superheated water vaporizes quickly because of pressure drop, resulting in the product expanding and cooling rapidly. A decrease in temperature stabilizes the bubble structure, and a puffed; the crispy structure is obtained. However, the product's water content has to be so low that the glass transition temperature of the product is higher than ambient temperature to maintain the crispy structure. In some cases, extrudates must be dehydrated after extrusion to obtain sufficiently low water content (Yağcı et al., 2014).

## 3 Food Extrusion Equipment

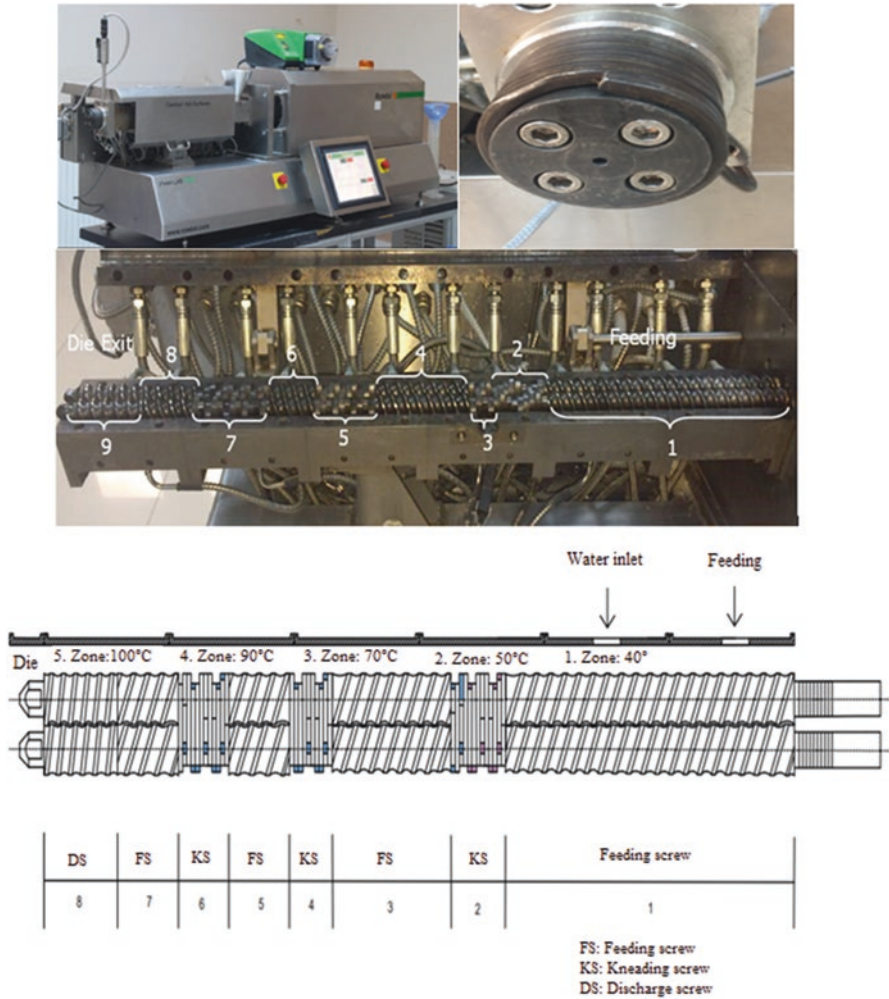
Several designs of extruders are competing in the market place. Single-screw and twin-screw extruders are the main types and widely employed in commercial food production. The main components of both extruders are raw material feeder, barrel, screw, and die. The raw materials are fed into the barrel and/or preconditioner of the extruder at a uniform rate controlled by a metering device, named as a feeder. In extrusion, usually, two feeders are needed: one feeder for solids (e.g., screw feeder), and one feeder for liquid (e.g., pump) (Yağcı et al., 2014). The solid ingredients used in food extruders have a relatively small particle size, particularly in powder form (Yacu, 2012). An essential feature of the preconditioner is its delivery of uniformly pretreated ingredients to the extrusion screw's feed section. The preconditioner is either an atmospheric or a pressurized chamber in which raw granular food ingredients are uniformly moistened and/or heated by contact with water or live steam (Harper, 1986). Most extruders operate with temperature control at the different barrel segments, named as barrel zones. Barrel heating generates conductive and convective heat in the filled and partially filled zones. The proportion of each heat source depends on the physical (i.e., specific heat, phase transition temperature, moisture content, density, particle size, and gelatinization enthalpy) and the feed's

rheological properties, barrel temperature profile, and the motor power applied. To prevent material from burning on the hot barrel surface, or inhibit excessive browning, or limit the degree of protein denaturation, the barrel jacket can be fed with chilled water (Yağcı et al., 2014).

A single-screw extruder is composed of one screw, while there are two rotating screws (co- or counter) in a twin-screw extruder. Single-screw extruders were the first types used in the food industry and continue to have wide use in products like pasta and other cereal products. Their operation is relatively simple. Because single-screw extruders have relatively poor mixing ability, they are often used with materials that have been either premixed or preconditioned. Twin-screw extruders consist of two screws of equal length placed inside the same barrel. One major difference between single-screw and twin-screw extruders is the type of transport in the extruder. Single-screw extruders depend entirely on frictional drag flow in the solids conveying zone and viscous drag flow in the melt-conveying zones. The transport in a twin-screw extruder is less dependent on the material's frictional properties due to the action of the second screw in the intermeshing region (Yacu, 2012). Thus, the feeding mass can be transported effectively further in the barrel and finally out of the extruder due to the sweeping phenomenon of the twin-screw. This is an important property, especially when sticky material is extruded (Riaz, 2001). For this reason, twin-screw extruders have found a wide application in the food industry due to their better process control and versatility, flexible design permitting, easy cleaning, rapid product change, and ability to handle a wide variety of formulations (Rokey, 2000; Riaz, 2001).

Twin screws extruders are generally categorized according to the direction of screw rotation (co-rotating and counter-rotating) and the degree to which the screws intermesh. In the counter-rotating position, the extruder screw rotates in the opposite direction. These types of extruders are not widely used in the food industry. They are good at processing relatively non-viscous materials requiring low speeds and long residence times, such as gum and jelly (Riaz, 2001). In the co-rotating extruders, screws rotate in the same direction (Fig. 36.1). These types of extruders are most commonly used in the food industry, especially in snack food production, because of their high capacity and enhanced mixing capability, good control over residence time distribution, self-cleaning mechanism, and uniformity of processing (Harper, 1986; Riaz, 2001).

Twin-screw extruders can also have intermeshing screws in which the flights of one screw engage the other. Intermeshing co-rotating extruders are particularly suited to applications where a high degree of heat transfer is required but not forced conveyance and are thus widely used to produce expanded products (Ainsworth & Ibanoglu, 2006). Several screw elements can be assembled on an extruder shaft. Functionally, some elements convey raw and preconditioned material into the extruder barrel, while other elements mix and compress the feedstock (Fig. 36.1). Where kneading is required, there may be one or several mixing blocks in the screw (Burtea, 2001). Screw geometry, speed, and the addition of reverse screw elements increase shear and control energy distribution responses such as melt temperature, torque, and pressure, and the melt temperature then determines the degree of puffing



**Fig. 36.1** Co-rotating intermeshing twin-screw extruder (Rondol Technology, England) and typical screw configuration for the expanded snack

(Sokhey et al., 1997). For a twin-screw extruder, the options for screw geometry and range of screw profile are numerous. Parameters that can be varied in a twin-screw extruder include the screw's pitch, number of flights, root diameter (diameter of the screw without any flights), angle of pitch of the screw, degree of intermeshing of the two screws, and presence and type of mixing elements. Screw speed typically falls in the range of 100–500 rpm. The normal minimum screw speed range is 70–100 rpm. Below this, the volumetric capacity would be severely limited and make the majority of food extrusion products costly to manufacturers.

The extruder die plays an essential role in the extrusion process. Increasing the die resistance to the melt flow by reducing the number of die outlets or decreasing

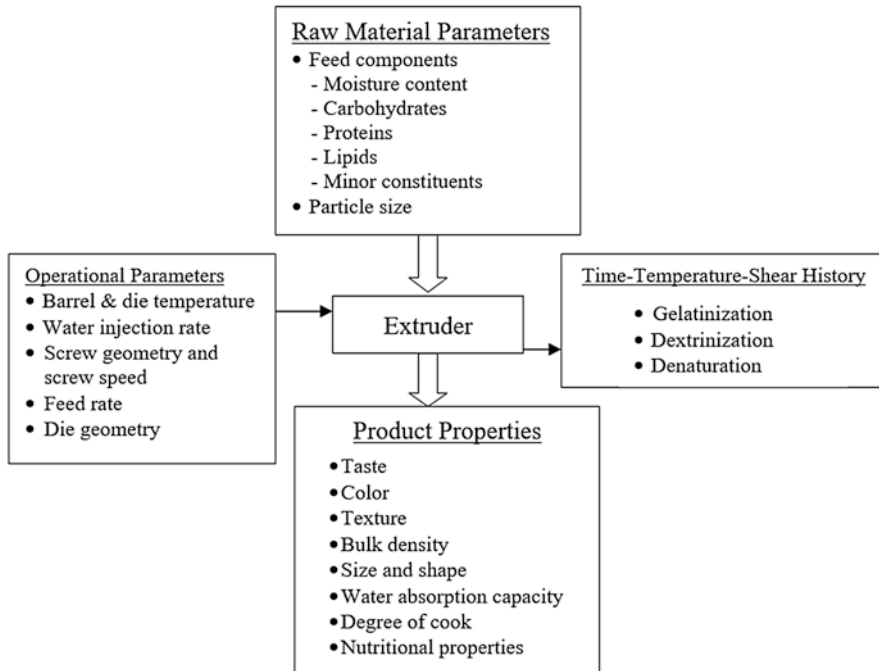
the opening's physical dimensions would generate a higher pressure drop across the die. The extruder die determines the dimensions of the semi-finished products. There may also be many holes in the die. Die-face cutting with a rapidly moving knife blade may be used to produce specific pieces of the extrudate. The die's construction and shape are important to give uniform flow and correct dimensional size (Sokhey et al., 1997; Harper, 1986). A die with a small diameter and shorter length would be used for greater radial expansion (Sokhey et al., 1997).

## 4 Extrusion Process Parameters

It is necessary to measure important process parameters to know what is going on in the extruder and to be able to control the whole process and, eventually, the product properties. Control of extrusion processes is difficult due to strong interactions between mass, energy, and momentum transfer, coupled with complex physico-chemical transformations, which govern final product properties (Chessari & Sellahewa, 2001). The formulation of the feed controls the quality of extruded products, pre-extruder operation (blending and preconditioning), extruder screw geometry, L/D ratio, die design, extruder operating conditions (feed rate of solids and liquid into the barrel, the temperature of the die and/or barrel zones and screw speed) and post extruder operations (drying, toasting and flavor addition). The conditions under which extrusion systems process a feed include both independent and dependent variables. Independent operating variables include raw material formulation, feed rate, barrel temperature profile, and screw/die geometry, which the extruder operator can directly control. Changes in the operating parameters will cause changes independent process variables such as die temperature, die pressure, residence time and viscosity, as well as product quality, attributes through changes in specific mechanical energy (SME), and thermal energy inputs during the mass residence in the extruder (Fig. 36.2). The most important operational parameters are die pressure, die temperature and motor torque. They are the best indicators of how well or how poorly extruder functions and are usually used as measured process outputs to indirectly monitor product quality.

## 5 Effect of Extrusion on Nutritional Value

Maintaining and increasing the nutritional quality of food during processing always important for the food processors. Extruded foods have been proven to provide nutritious products, combine quality ingredients and nutrients to produce processed foods that contain precise levels of each required nutrient (Cheftel, 1986). Nutritional concern about extrusion cooking is reached at its highest level when extrusion is



**Fig. 36.2** Extrusion process parameters and their interactions. (Reprinted from Yağcı et al., 2014 with permission)

explicitly used to produce nutritionally balanced or enriched foods, like weaning foods, dietetic foods, and meat replacers (Singh et al., 2007). In many cases, long-term heat treatments lower the nutritional value of food products. Extrusion is a high-temperature short time process. The cooking temperature can be as high as 180–190 °C during extrusion, but residence time is usually only 20–40 s (Riaz, 2001). Shortness of heat treatment may prevent the formation of color or aroma compounds during extrusion. Therefore, extrusion process parameters have to be adjusted, so that desired changes are occurring, but undesired changes are restricted (Yağcı et al., 2014). With improvements in starch and protein digestibility, prevention or reduction of nutrient destruction is of importance in most extrusion applications. Five general chemical and physicochemical changes can occur during extrusion cooking: binding, cleavage, loss of native conformation, recombination of fragments, and thermal degradation. Anti-nutritive compounds can be reduced during extrusion to provide safer and more nutritious foods (Camire, 2001). The effects of extrusion on the nutritional value of extruded foods are summarized in Table 36.1.



**Table 36.1** Effect of extrusion on the nutritional value of extruded food

Component	Effect of extrusion on nutritional value
Protein and amino acids	<ul style="list-style-type: none"> <li>• <b>Denaturation:</b> Thermo-mechanical energy applied during extrusion cooking causes protein macromolecules to lose their native structure as they form a viscoelastic mass (Riaz, 2004). Denaturation results in decreased protein solubility and improved digestibility (Steel et al., 2012). Enzymes lose their biological activity following exposure to high temperature and shear in the extruder (Ramachandra &amp; Thejaswini, 2015).</li> <li>• <b>Improve digestibility:</b> During extrusion, disulfide bonds are broken and may re-form. Electrostatic and hydrophobic interactions favor the formation of insoluble aggregates. The creation of new peptide bonds can dissociate into smaller subunits. Exposure of enzyme susceptible sites improves digestibility (Camire, 2000).</li> <li>• <b>Reduce the availability of amino acids:</b> Extrusion processing may influence reductions in albumins, globulins, prolamins, and glutelins in the final product (Moscicki et al., 2013). High barrel temperatures and low moistures promote Maillard reactions during extrusion. Reducing sugars produced during the extrusion process may react with the free amine groups of lysine and other amino acids. Low moisture content and high-temperature process conditions favor the reaction between amino acids and reduce sugars, leading to colored compounds' development. Maillard reactions between protein and sugars decrease the protein quality, depending on the types of raw material, composition, and processing conditions (Steel et al., 2012).</li> <li>• <b>Destruct anti-nutritional factors:</b> Destruction of trypsin inhibitors, haemagglutinins, tannins, and phytates, all of which inhibit protein digestibility (Singh et al., 2007). Extrusion was established as the most effective processing method for the destruction of the antinutritional factors; phytic acid (99.3%), trypsin inhibitors (99.5%), and tannins (98.8%) in lentils, without altering protein content (Rathod &amp; Annapure, 2016).</li> </ul>
Starch	<ul style="list-style-type: none"> <li>• <b>Improve digestibility:</b> In the case of starch component, passage through the extruder barrel shear amylopectin branches, but both amylose and amylopectin undergo reductions in molecular weight. The starch depolymerization that occurs during extrusion can be exploited to increase the digestibility of foods (Camire, 2012). Extrusion increased the enzymatic availability of starch through gelatinization, inactivation of endogenous <math>\alpha</math>-amylase inhibitor, disruption of cellular structure, size reduction, and increased starch surface, partial separation from bran and protein (Cheftel, 1986).</li> <li>• <b>Produce resistant starch:</b> Extrusion has been evaluated as a means to produce RS starch; the reaction with acids also favors RS formation during extrusion (Camire, 2012). The branches of amylopectin molecules could react with other carbohydrates in novel linkages that cannot be digested by digestive enzymes (Camire, 2001).</li> <li>• <b>Formation of amylose-lipid complex:</b> Extrusion cooking is responsible for changing the extent of molecular associations between components, e.g., the amylose-lipid complex. Lipids form amylose-lipid complexes during extrusion cooking, which modifies the products' physicochemical properties, including expansion ratio, bulk density, and water solubility index (Bhatnagar &amp; Hanna, 1996). The formation of the amylose-lipid complex is reported to retard the rate of carbohydrate utilization in the gut (Hagenimana et al., 2006).</li> </ul>

(continued)

**Table 36.1** (continued)

Component	Effect of extrusion on nutritional value
Lipid	<ul style="list-style-type: none"> <li>• <b>Reduce fat content:</b> Some fat may be lost as free oil due to complexes with amylose or protein (Camire, 2000).</li> <li>• <b>Lipid oxidation:</b> Formation of porosity in expanded products, leading to the increased surface area. Lipolytic enzymes and other enzymes that promote oxidation may be inactivated during extrusion, and starch-lipid complexes formed may be more resistant to oxidation (Camire, 2001). The results of the few studies have demonstrated that DHA and EPA fatty acids are stable through the extrusion process (Camire, 2012).</li> <li>• <b>Formation of amylose-lipid complex</b></li> </ul>
Dietary fiber	<ul style="list-style-type: none"> <li>• <b>Increase in soluble DF content:</b> Extrusion cooking does not significantly change DF content, but it solubilizes some fiber components. This has been attributed to the cleavage of non-covalent and covalent bonds between fibers and other molecules, with the resultant smaller molecules being more soluble (Steel et al., 2012). DF content tends to increase, mainly due to increases in soluble DF and enzyme-resistant starch fractions at more severe conditions (Singh et al., 2007). Extrusion cooking can modify DF-rich materials for use as nutritious food ingredients or as biofuels (Camire, 2012).</li> </ul>
Vitamins	<ul style="list-style-type: none"> <li>• Vitamin losses are usually minimal when compared to other traditional thermal treatments because of high-temperature short time process and rapid cooling after extrusion (Offiah et al., 2019).</li> <li>• <b>Water-soluble vitamins:</b> Loss of heat-sensitive vitamins is expected during thermal treatments at 100 °C and above, especially for water-soluble vitamins such as vitamin C (Moscicki et al., 2013). Cereals constitute an important source of B-vitamins. Thiamine is the water-soluble vitamin most susceptible to thermal processing. Riboflavin appears to be retained well, whereas thiamine retention seems to be highly dependent on process condition. Niacin, pyridoxine, and folic acid appear to be comparatively stable during extrusion cooking (Asp &amp; Bjorck, 1989).</li> <li>• <b>Fat-soluble vitamins:</b> Vitamin D and K are reasonably stable. Vitamins A and E and their related compounds, carotenoids, and tocopherols are not stable in the presence of heat (Camire, 2000).</li> </ul>
Minerals	<ul style="list-style-type: none"> <li>• <b>Improve absorption of minerals:</b> Mineral absorption may be improved by extrusion due to the destruction of inhibitory factors like condensed tannins and phytates (Steel et al., 2012). Extrusion hydrolyses phytate to release phosphate molecules (Singh et al., 2007).</li> </ul>
Phytochemicals	<ul style="list-style-type: none"> <li>• <b>Cause reduction in total phenolics:</b> The most important groups of phytochemicals found in whole grains can be classified as phenolics, carotenoids, vitamin E compounds, lignans, <math>\beta</math>-glucan, and inulin (Liu, 2007). It might be possible that lost phenolics reacted with themselves or other compounds to form larger insoluble materials (Camire, 2000). Extrusion caused a reduction in total phenolics of extruded oat cereals by 24–46% (Viscidi et al., 2004). Natural phenolic compounds added to grains before extrusion cooking may synergize and protect the endogenous antioxidants.</li> <li>• <b>Loss of anthocyanins:</b> Significant losses are reported for anthocyanins during extrusion cooking, mainly due to the high temperature employed because they are quite sensitive to high temperature (Alam et al., 2016). Anthocyanin losses of low magnitudes (10%) are also reported for extrusion cooking of blueberry, cranberry, raspberry, grape powders, and corn blends (Camire et al., 2007), and losses up to 32% are also reported for blueberry-corn blends (Chaovanalikit et al., 2003).</li> <li>• <b>Other phytochemicals:</b> Lycopene, ascorbic acid, and major flavonoids are heat-labile and degrades rapidly at temperatures higher than 100 °C. Consequently, greater loss happens as the severity of the extrusion process is increased. Besides, screw wear due to high shear conditions could significantly increase iron concentration in the extrudate, resulting in greater destruction of L-ascorbic acid (Camire, 2012; Alam et al., 2016).</li> </ul>

## 6 Characteristics of *Opuntia* spp. as a Raw Material

*Opuntia* spp., called “prickly pear” or “cactus pear”, is an emergent fruit crop that evolved commercially during the second half of twentieth century. Cactus pear is an essential resource for semiarid zones. Although this species is native to Mexico, it has spread and been cultivated across the world. Generally, the genus *Opuntia* consisting of about 200 species, thrives in South Western USA, Mexico, and Mediterranean climates (Patel, 2013; Melgar et al., 2017). The fruit of prickly pears, commonly called cactus fruit, cactus fig, Indian fig, nopales, or tuna in Spanish, is edible. They are consumed as roasted, cooked, boiled or blended (juice) as a beverage (Peña-Valdivia et al., 2012). Although it could be used as a forage, cactus pear fruit is primarily consumed as a fresh commodity (Sáenz et al., 1998). Currently, prickly pear is the most heavily commercialized fruit in Mexico, globally producing 70% (Obón De Castro et al., 2010). Ramírez-Rodríguez et al. (2020) give a detailed description of the edible fraction of fruit composition, forming 57% of the whole fruit, contained 89.3% water. They noted that prickly pear composed of 10.4% carbohydrate, 4.65% fiber, and 0.84% protein. Moreover, it has a high content of potassium (220 mg), followed by magnesium (85 mg), calcium (56 mg), vitamin C (14 mg), and niacin (0.46 mg). *Opuntia* contains a range of bioactive compounds in variable quantities, such as phenolics, dietary minerals, and betalains (Butera et al., 2002; Guzmán-Maldonado et al., 2010). The bioactive compounds described are ascorbic acid, pectins,  $\beta$ -carotene, betalains, and phenolic compounds, mainly phenolic acids and flavonoids (Melgar et al., 2017). The fibers, minerals, and antioxidants-rich *Opuntia* pears are touted as ideal sources of nutraceuticals and functional food. Cactus pears have also been recognized for their pharmacological properties. Many indigenous communities, such as Pima Indian, Tunisian, Mexican, and Chinese, have used pears as ethnomedicine for various ailments *viz.* asthma, inflammation, ulcer, and diabetes (Patel, 2013). The composition of the fresh cactus pears varies according to cultivation types and climatic conditions. Variability is expressed in all parts of the plant, fruits included. Features like skin and pulp color, pulp texture, sugar content, and juice acidity are directly related to the presence, intensity, and activity of nutritional and functional compounds (Yahia Elhadi & Mondragon-Jacobo, 2011).

Cactus pear mostly stand out with its high sugar, betalain pigments, and fiber contents. The fruit has a wide range of color due to betalain pigments, nitrogen-containing heterocyclic pigments present in a limited number of families of the plant order *Caryophyllales*. The water-soluble pigments of cactus pear are localized in the plant vacuole (Stintzing et al., 2001; Osorio-Esquivel et al., 2011; Melgar et al., 2017). The most important betalains in cactus are betacyanins and betaxanthins (Osorio-Esquivel et al., 2011). Betalains possess higher molar absorption coefficients in the visible light spectrum than anthocyanins (Stintzing et al., 2001). The coloring properties of the Betalain pigments make the cactus pear suitable for food colorant production, substituting the synthetic dyes (Patel, 2013). The nutritional and health benefits of cactus pears are associated with their antioxidant

properties related to ascorbic acid, phenolic compounds, and a mixture of yellow betaxanthin and red betacyanin pigments, which have strong radical-scavenging and reducing properties (Yahia Elhadi & Mondragon-Jacobo, 2011).

The cactus pear's total antioxidant activity was twofold higher than that of pear, apple, tomato, and grape and similar to that of red grape raisin, orange, and grapefruit (Livrea & Tesoriere, 2006). The phytochemical constitution of cactus pears has been reviewed comprehensively in the study of Patel (2013). They reported that ferulic acid is the chief component of the total phenolic compounds. The flavonoid fraction consisted of rutin and isorhamnetin derivatives. Phenolic compounds like anthocyanins, phenolic acids, stilbenes, and tannins were also reported. Ramírez-Rodríguez et al. (2020) outlined the most abundant phenolic acids of cactus pear as piscidic, hydroxybenzoic, protocatechuic, ferulic, vanillic, *trans*-coumaric, and *trans*-cinnamic acids. It was reported that the presence of phenolic acids was high in peels, wherein the total phenolic content in the peel was higher than in the pulp. Seven phenolics were identified as protocatechuic, 4-hydroxybenzoic, caffeic, vanillic and syringic acids, rutin, and quercetin for sour prickly pears (Osorio-Esquivel et al., 2011). Methanol extracts and semi-purified fractions A (phenolics and flavonols) and B (betacyanins) of sour prickly pears showed high antioxidant activity mainly in the pericarp. In another study, Sicilian red, yellow, and white cultivars of *Opuntia* pear were analyzed for biothiols, taurine, and flavonols, as well as tocopherols and carotenoids. The yellow cultivar showed the highest level of reduced glutathione, whereas the white cultivar showed the highest cysteine content (Tesoriere et al., 2005). The antioxidant compounds in extracts from four cactus fruit varieties were investigated by Kuti (2004). Quercetin was found to be the most abundant in all varieties examined. The fruit extracts' antioxidant activity was stronger in the purple-skinned than other varieties, and this result was consistent with total flavonoid content. It was concluded that cactus fruits are a rich source of natural antioxidants for food, and the antioxidant capacity of cactus fruits may be attributed to their flavonoid, ascorbic acid, and carotenoid contents.

Cactus pear also a good source of soluble sugars, with glucose and fructose being the predominant carbohydrates (Stintzing et al., 2001). It was reported that the Chilean varieties of the pears are the most desirable owing to their high sugar and low seed contents. Mexican varieties with high yields do not contain much sugar (Patel, 2013). Melgar et al. (2017) measure the soluble sugar content and the total carbohydrate content of the cactus pear's different cultivars. They found that pears have higher content in carbohydrates and lower sugar content, indicating that most carbohydrates could be fibers or longer polysaccharide chains like mucilage. They concluded that the mucilage properties could also be potentially used by food, pharmaceutical, and cosmetic industries as thickener agent. The usage of cactus pear juice to prepare natural sweetener was reported (Sáenz et al., 1998; Patel, 2013).

The studies related to the fiber content of the *Opuntia* spp. are mostly about polysaccharides from different parts of the plant tissues is on mucilages and pectins. The mucilages in *Opuntia* tissue are responsible for water retention and can be used as dietary fiber or food thickening agents (Stintzing et al., 2001). Mucilage, pectin, and cellulose were purified from the fruits of *O. Matudae*, indicating their richness

in soluble and insoluble dietary fibers (Armenta & Peña-Valdivia, 2009). The pulp of the cactus pear fruits contains are reported to be rich in dietary fibers and natural antioxidants (Bensadón et al., 2010). El Kossori et al. (1998) found that *Opuntia ficus-indica* pears contained mostly ethanol-soluble carbohydrates in their composition. The pulp contained glucose and fructose, while the skin essentially contained glucose. The pulp fibers were rich in pectin while the skin and seeds were rich in cellulose. Garcia-Amezquita et al. (2018) reviewed some studies about the dietary fiber content of *Opuntia* fruits. They specified that cactus fruit peels have 7.0–8.5 of SDF, 31.2–32.6 IDF, and 38.1–41 of TDF contents (Garcia-Amezquita et al., 2018; Tejada-Ortigoza et al., 2017). In another study (Diaz-Vela et al., 2013), cactus pear flours, obtained by drying and grinding of *Opuntia ficus-indica* peel pulp, had SDF content of 33.9%, IDF content of 37.0%, and TDF content of 71.0%, indicating the possible usage of cactus fruit as a source of dietary fiber in the food formulations. Wastes of *Opuntia matudae* pears were analyzed against TDF content. It was reported that the discarded peel's total fiber content was twofold higher than that of edible mesocarp (Guzman-Maldonado et al., 2010). Based on the available data in the literature, cactus pear fruit can be considered an excellent dietary fiber source.

## 7 *Opuntia* spp. Extruded Food Products

Current extrusion studies have usually been related to functional food products using legumes, vegetables, and cereals as well as fruits end their extracts because of the consumer demand increasing day to day in this area. There is limited information in the literature about applying the extrusion process for the development of cactus-based products using pears and cladodes, even the less from the processing of cactus fruit using any food process. Up to the present time, the studies were usually made on the incorporation of cactus fruit/seed/pulp on cereals and production of the expanded-type food products using extrusion. Table 36.2 shows a summary of applications for the extrusion process utilizing the *Opuntia* spp. These products outrival with their structural characteristics, while this technology protects bioactive and functional compounds due to the HTST process conditions. Several factors like extruder processing conditions, e.g., screw speed, barrel and die temperatures, feed rate, and the composition of the blended formula and type of the ingredients used, were investigated in these studies to observe/examine the final product quality.

One of the first studies demonstrating the possibility of utilization of cactus fruit in the extrusion process was investigated by El-Samahy et al. (2007). They used concentrated fruit puree (40°Brix), obtained from orange-yellow fruit and red-colored fruit, to value add to the rice extrudate. The cactus fruit puree was added to rice flour at five levels (0%, 5%, 10%, 15%, and 20%), and their effects on physical, nutritional, and sensory attributes of extrudates were investigated. As the puree amount increased in the formulation, physical and textural properties were weakened, showing the difficulty of blending at a higher substitution level. Substitution levels of 5%, and 10% of concentrated fruit puree gave the best results for extruded

**Table 36.2** Applications for the extrusion process utilizing *Opuntia* spp.

Source	Raw material	Extruder Type	Objective	Reference
Orange and Red cactus pear fruit	Rice flour	Single screw extruder	Utilization of cactus fruit in extruded food product formulation	El-Samahy et al. (2007)
<i>Opuntia</i> pulp from prickly pear fruit	Rice flour	Twin-screw co-rotating extruder	Searching extrusion potential of prickly pear fruit	Setia (2010)
Cactus pear fruit pulp	Rice flour	Twin-screw co-rotating extruder	Utilization of cactus fruit in extruded food product formulation	Sarkar et al. (2011)
Yellow-orange fruit from <i>Opuntia ficus-indica</i>	Rice grit Corn grit	Single-screw extruder	Measurement of stability of the flavonols towards the extrusion of extrudates fortified with the cactus fruit	Moussa-Ayoub et al. (2015)
Encapsulated powder of red cactus pear juice	Corn starch	Twin-screw co-rotating extruder	Evaluation of the effect of extrusion conditions on bioactive compounds of encapsulated red cactus pear powder.	Ruiz-Gutierrez et al. (2015)
Encapsulated powder of red cactus pear juice	Maize grit	Twin-screw co-rotating extruder	Evaluation of the effect of encapsulated powder of the red cactus pear on extruded cereals' physicochemical properties.	Ruiz-Gutierrez et al. (2017)
<i>Opuntia dillenii</i> seed	Rice grit	Single screw extruder	Enrichment of extruded products with fiber and antioxidants	Rayan et al. (2018)

products with good functional, nutritional, and sensory characteristics. Setia (2010) investigated the use of red-colored prickly pear fruit to formulate the extruded product. Firstly fruit juice was prepared from red pear fruit, and seeds were removed. The prepared juice was blended with rice flour at ratios of 1: 10, 1:8, and 1:6 (rice flour: prickly pear pulp). After drying and grounding of the blends, they were extruded using a twin-screw extruder (screw speed: 400 rpm, feed flow rate: 20 kg/h, feed moisture content: 13%, die diameter: 4.5 mm, extruder length: 1000 mm, and L/D ratio: of 40:1). According to the results of this study, the addition of *Opuntia* pulp solids decreased true density, porosity, radial, axial, and overall expansion ratios of the extrudates, while it increased apparent density and breaking strength. Similarly, prickly pear fruit has been investigated to develop a rice-based expanded extruded product (Sarkar et al., 2011). Material formulations of rice flour and cactus pear fruit pulp (rice flour solids: pure solids were 6:1, 8:1, and 10:1) were extruded using a twin-screw extruder at 15 kg/h feed rate, 13% (w/w) feed moisture, 400 rpm screw speed, and 40:1 L/D ratio. The extruder's temperature profile from feed to die exit was maintained as follows: 25, 30, 40, 50, 60, 70, 80, 100, 120, 140 °C. Response variables of true and apparent density, porosity, expansion indices, and textural properties were measured for extruded products. An increase in cactus pear fruit

level increased breaking strength and apparent density of products; however, porosity and radial expansion ratio decreased with an increase in fruit solid level. This study indicated that peeled prickly pear could be effectively utilized as a food ingredient for the production of expanded extruded food products and increase the overall fruit utilization.

Moussa-Ayoub et al. (2015) claimed that isorhamnetin glycosides are the dominant flavonol derivatives occurring in the peels and cladodes of cactus *Opuntia ficus-indica* fruits and might be used as markers for analyzing the authenticity of food products containing cactus fruits. They investigated the use of whole yellow-orange fruit from cactus as an ingredient of rice- and corn-based snacks. In their study, the impact of extrusion cooking on the flavonol profile was characterized in rice- and corn-based extruded products fortified with a freeze-dried preparation from whole cactus fruit. The freeze-dried fruit preparation was added in ratios of 0%, 2%, 6%, and 10% into each formulation. Extruder operation conditions were selected as relatively mild. The temperatures of the single screw extruder feed, cooking, and die zones were set at 100 °C, 140 °C, and 160 °C, respectively. The screw speed in feeding and cooking zones and screw compression were 160 rpm, 250 rpm, and 4:1, respectively. Flavonols of extrudates were tested using the HPLC-DAD system, and antioxidant activity using DPPH assay was also measured. Analysis of the final extrusion products showed that flavonol profiles from the fortified rice or corn products, even at the lowest level of addition (2%), were similar to the fruit's original profile before processing. Furthermore, the total flavonol content in extruded products was found to be not affected by extrusion cooking. The authors suggested that the unique flavonol profile of fruits from cactus might serve as a biochemical marker to evaluate the authenticity of products made from whole cactus fruit or the fruit's peel.

As mentioned above, the cactus pear has significant potential as a colorant in food due to betalains' presence. Ruiz-Gutierrez et al. (2015, 2017) published series of studies about the use of encapsulated powder form of the red cactus pear in the extrusion process to reveal the possibility of using it as a pigment source in food formulations. In the first study, corn starch and encapsulated powder (2.5% w/w, obtained by spray drying) were mixed and processed by extrusion at different barrel temperatures (80, 100, 120, and 140 °C) and screw speeds (225, 275, and 325 rpm) using a twin-screw extruder. Color properties, total phenolic content, antioxidant activity, betalain content, betacyanin retention, and mean residence time were measured for extruded products. Increases in barrel temperature and screw speed decreased mean residence time. This study showed that shorter exposure time of the components of interest to high-temperature conditions, providing greater retention of total polyphenol, betacyanins, and betaxanthins, as well as antioxidant activity. However, a significant reduction in the total phenolic content and antioxidant activity was observed compared to the initial content of these components in the raw mixture. They also reported pigment degradation of betalains in encapsulated red cactus pear powder as a function of temperature (80, 100, 120, and 140 °C) during an extrusion process. After that, Ruiz-Gutierrez et al. (2017) blended encapsulated powder of the red cactus pear (2.5%, 5.0%, and 7.5%, w/w) with maize grits and



**Fig. 36.3** Extruded cereal pigmented with different content of encapsulated powder of red cactus pear; (a) 7.5%, (b) 5.0%, (c) 2.5%, and (d) 0%. (Reprinted from Ruiz-Gutierrez et al., 2017, Use of red cactus pear (*Opuntia ficus-indica*) encapsulated powder to pigment extruded cereal. Journal of Food Quality, with permission)

extruded (Fig. 36.3). The extrusion conditions used during experimentation were as follows: feed water (0.22 kg water/kg dry matter), barrel temperature of 100 °C, and a screw speed of 325 rpm. In this study, the physical, chemical, and sensory characteristics of the extruded cereal were evaluated.

The characteristics positively affected by an increased amount of encapsulated powder were water absorption index, milk absorption, color parameters, antioxidant activity, total phenolics, total betacyanin, and total betaxanthin content. However, density and texture were negatively affected by the increasing amount. As shown in Fig. 36.3, the use of red cactus pear encapsulated powder changes the color of the final extruded product, demonstrating the power of the betalain pigments for color production. They noted that encapsulated powder has a high betalain content, which has colors with a greater red, purple characteristic. Researchers observed that panelists chose the extruded cereal containing 2.5% red cactus pear encapsulated powder because its characteristics were similar to the control but had better color.



Later, Rayan et al. (2018) investigated the incorporation of powder of cactus *Opuntia dillenii* seeds to enrich rice-based extrudates in terms of fiber and antioxidants. In this study, *Opuntia dillenii* seed powder, separated, washed, and dried from cactus fruit, blended with rice grits in ratios of 0 (control), 2%, 4%, 6%, 8%, 10%, 15%, and 20%, and the blend was extruded. The extrusion cooking process for the prepared formulas was undertaken using a single-screw extruder at pre-determined optimum processing conditions. The optimum extrusions conditions were different in feeding and cooking zones. The adjusted conditions for feeding and cooking zones were 160 rpm, and 250 rpm for screw speed, 100 °C, and 180 °C for barrel temperature. The products were shaped using 3 mm round die, and the temperature was set at 180 °C. The authors tested the final extruded products in chemical composition, functional properties, color attributes, antioxidant activity, and sensory acceptability. They observed that the seeds of *Opuntia dillenii* contained total phenolics of 39.5 mg/100 g, flavonoids of 595 mg/100 g, and antioxidant activity of 7.47% or 108 µmol trolox/100 g. Incorporating the seeds in the rice-based extrudates greatly enhanced the levels of total phenolics up to fourfold, total flavonoid contents up to threefold, and antioxidant activity up to threefold on the level of seed powder. As the incorporation of seed powder increased, the crude fiber content extrudates significantly increased from 0.22 for the control formula to 8.04 g/100 g for a formula containing 15% seed powder. It was concluded that *Opuntia dillenii* seed powder could be incorporated in rice to develop snack products of acceptable functional, nutritional, and sensory properties. The best acceptability for rice snacks enriched with *Opuntia dillenii* seeds at 15% was observed.

## 8 Conclusion

Further studies are needed to exhibit the functional properties and health-promoting effects of the different extruded food products produced by incorporating cactus-based ingredients. The extruder can be considered a reactor of the HTST process that can accelerate chemical reactions among the components present in some ingredients. The extruder system could be controllable in many parameters like temperature, moisture, feed rate, etc. The chemical reactions can also be provoked by a distinct biochemical or chemical component like an enzyme or a pH controlling agent. Any food producer/designer could operate under optimum conditions during the transformation of raw ingredients to processed materials to create newly formed compounds/ingredients in the extrusion process. The *Opuntia* plant stands out with its bioactive compounds, especially betalain, colored pigments, flavanols, and phenolic substances, as well as fiber content. In this respect, the extruder system could be used as a reactor for purification/extraction/synthesis of these bioactive compounds from *Opuntia* spp. At this moment, the functional food market could be enriched with a category of high-quality products obtained from *Opuntia* spp.

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# Chapter 37

## Industrial Uses of *Opuntia* spp. By-products



Juan Carlos Guevara-Arauz

**Abstract** Prickly pear cactus stems, better known as Nopal (*Opuntia* spp.), is spread around the world as feedstock. It has multiple functional compounds which could be applied in functional foods or as source of nutraceuticals. Nopal application in sugar-based confectionery, bakery and dairy products improved the quality and sensorial attributes and provided bio-functional activities. In addition to applications in pharmaceuticals industry, the stems have been used since ancient time mainly as cattle fodder, fence, to purify water and to restore or control erosion of arid and semiarid lands. As cattle fodder, solid-state fermentation of stems increased the crude protein content up to 26%. As coagulant-biofloculant nopal biopolymers are applied to water treatment to remove turbidity, suspended solids, organic carbon, kaolin, lead, arsenic, heavy metal ions, pesticide, dyes, and bacteria. Restoration of natural ecosystems with nopal produces besides a tolerant forage to drought conditions, ecological benefits like carbon capture and decreasing the global warming. Another application of nopal is to improve house paint. It is friendly with environment and works for waterproofing. Natural dyes present in fruits, like betalains, are used as natural food colorants. The mucilage of *Opuntia* spp. has been used to fix colors of dyed fabrics. The stems and their polysaccharides could be used in (a) bio-nano-packaging for the elaboration of coatings and biodegradable-edible films to extend shelf life of fresh, frozen and processed food, and (b) vegan leather with adequate softness. The stems also could be used as a source of enzymes in the dairy sector. In construction, cactus provides benefits in adobes, mortars and concrete reinforcing steel, improves water absorption, enhances freeze-salt resistance, and delays corrosion. In restoring historical buildings and monolith, nopal is used for impregnation of minerals (consolidation). Nopal extracts, mucilage and pectin are used as redox agent to synthesized metal nanoparticles (Li, Ag, Au, hydroxyapatite and  $ZnFe_2O_4$ ). Nopal has been assessed as resource to generate biofuels: bioethanol from lignocellulosic material, biogas from polysaccharides, biodiesel from seed oils, and electricity from nopal biogas effluent. An electrochemical cell has been fabricated using cactus stems as an electrolyte. Betacyanin from fruit and aerobic

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fermented nopal extracts showed photosensitizer properties with applications as dye-sensitized solar cell and holography. The industrial uses of *Opuntia* spp. stems are described in this chapter. The potential technological uses of these plant and its derivatives are also discussed.

**Keywords** Agro-industry · Waste · Soluble fiber · Pectin · Mucilage · Coagulant-bioflocculant

## 1 Introduction

In order to establish a relationship between scientific advances and the main investigations developed around the industrial exploitation of cactus, carried out around the world, this chapter describes the main uses and applications of nopal, as well as the results of the most outstanding investigations related to the comprehensive use of this horticultural product, which surprises us more day after day due to its multiple benefits and applications in several areas of the human development.

The use of nopal and by-products has been evidenced since pre-Hispanic times in Mexico, being a common practice in construction through its application to generate adhesion materials and paints; in water purification as a coagulating agent; and to obtain pigments present in cladodes (chlorophyll) and prickly pears (Betalains and Betacyanin).

Undoubtedly highlighting the increase in protein content (up to 26%) through solid fermentation, which strengthens the application of nopal as fodder for livestock.

It also amazing the use of biopolymers present in nopal for developing edible films and biodegradable packaging, both macro and nano structured, as well as in the green synthesis of various nanoparticles.

Likewise, in biofuels, significant progress has been made since bioethanol, biogas (methane) and electricity have been generated from cactus. The development of biofunctional foods, stands out without a doubt, it takes advantage of phytochemical content with both antioxidant and prebiotic activities present in cactus; which not only nourish but also have an impact on the oxidative status and therefore in consumer's health.

In this order of ideas, this chapter was structured as follows:

2. Fodder
3. Biological Fencing/Vegetative Barriers
4. Erosion Control
5. Water Treatment
6. Additive Used in Construction
7. Improving House Paint and General Paints
8. Nopal Mucilage in Edible Coatings and Packaging Applications
  - 8.1. Cactus Mucilage as a Coating Material

- 8.2. Cactus Mucilage as a Film Packaging Material
- 8.3. Development of Composite Materials Using Cactus Mucilage
9. Nanoparticles and Nanocomposites Based on *Opuntia ficus-indica*
  - 9.1. Cellulose Nanoparticles from *Opuntia* spp.
  - 9.2. Green Synthesis of Nanoparticles Using Cactus Pectin
  - 9.3. Green Synthesis of Nanoparticles Using Cactus Extracts
10. Bioenergy from Cactus
  - 10.1. Bioethanol
  - 10.2. Biogas Production (Methane)
11. Nopal as Source of Electricity
12. Nopal Used in Solar Cells and Halographic Materials
13. Bio-Functional Foods
  - 13.1. Tortillas
  - 13.2. Bread Rolls
  - 13.3. Juice and Beverage
  - 13.4. Ice Cream
  - 13.5. Salami

## 2 Fodder

*Opuntia ficus-indica* was introduced to continents in the eighteenth century by navigators with different aims: as vegetable to prevent scurvy, to make living fences, as a fodder to get carmine from cochineal, and as vegetable for human consumption (Diguët, 1928). The use of *Opuntia* for livestock feeding is an ancient practice and it has spread in countries like Algeria, Argentina, Bolivia, Brazil, Chile, Colombia, Spain, Mexico, Morocco, Israel, Italy, South Africa, Tunisia, USA, Peru and other countries (Santana, 1992). In some countries, the nopal is used all year round as emergency forage during drought (Table 37.1).

*Opuntia*'s advantages as forage include high biomass production, high efficiency in converting water into dry matter, good palatability, nutritional value, provide succulent forage during drought (evergreen), long drought resistance, tolerate severe use (high animal load), no adverse effects on animal health, low cost of establishments and maintenance, as well as soil and climate conditions adaptation (Ben Salem et al., 1996).

Due to its chemical composition, *Opuntia* does not constitute a complete food by itself, however, it is considered a good option to be used as fodder not only in arid and semiarid regions but also in stockbreeder farms throughout the year. The nopal during the dry season provides water (79–93%), 7–18% of protein (25–60 g/kg dry weight), 1.3–3% lipids (dry weight), and crude fiber 3.02–18.8% (dry base, good source of dietary fiber). The consumption of nopal as fresh food can contribute to

**Table 37.1** Nopal varieties used as fodder in different countries

Country	Variety	Source
UE	<i>O. lindheimeri</i>	Merrill et al. (1980), Migaki et al. (1969)
	<i>O. ellisiana</i>	Han and Felker (1997)
	<i>O. polyacantha</i>	Shoop et al. (1977)
Brasil	Los tres cvs.	Souza (1963)
	Redonda	Metral (1965)
	Gigante	Metral (1965)
	Miúda	Metral (1965)
	IPA 20	Santos et al. (1998)
Argentina	<i>O. spinulifera</i> Salm-Dyck	Guevara and Estévez (2003)
	<i>O. robusta</i>	Guevara and Estévez (2003)
	<i>Atriplex</i>	Guevara and Estévez (2003)
Tunez	<i>Acacia saligna</i>	Guevara and Estévez (2003)
Siria	<i>Atriplex halimus</i>	Guevara and Estévez (2003)
Mexico	<i>Opuntia leucotricha</i>	Bravo and Scheinvar (1995), Elizondo et al. (1987)
	<i>O. streptacantha</i>	Bravo and Scheinvar (1995), Elizondo et al. (1987)
	<i>O. robusta</i>	Bravo and Scheinvar (1995), Elizondo et al. (1987)
	<i>O. cantabrigiensis</i>	Bravo and Scheinvar (1995), Elizondo et al. (1987)
	<i>O. rastrera</i>	Bravo and Scheinvar (1995), Elizondo et al. (1987)
	<i>O. leptocaulis</i>	Bravo and Scheinvar (1995), Elizondo et al. (1987)
	<i>O. lindheimeri</i>	Bravo and Scheinvar (1995), Elizondo et al. (1987)
	<i>O. phaeacantha</i>	Bravo and Scheinvar (1995), Elizondo et al. (1987)
	<i>O. engelmannii</i>	Lopez García et al. (2003)
	<i>O. imbricate</i>	Lopez García et al. (2003)
	<i>O. microdasys</i>	Lopez García et al. (2003)
	<i>O. violacea (morado)</i>	Lopez García et al. (2003)
	<i>O. rufida (cegador)</i>	Lopez García et al. (2003)
Ecuador	<i>O. engelmannii</i>	Flores and Reveles (2010)
	<i>O. lindheimeri</i>	Flores and Reveles (2010)
Sudafrica	<i>O. robusta</i>	De Kock (2003)
	<i>O. fuscicaulis</i>	De Kock (2003)
	<i>O. ficus-indica</i> f. <i>inermis</i>	De Kock (2003)



35% of water demand from cattle under conditions that are not of extreme drought (Osorio et al., 2011). The minerals in nopal are calcium, potassium, magnesium, silica, sodium, and small amounts of iron, aluminum, and magnesium (Hernández-Urbinola et al., 2010).

*Opuntia* is a good source of water, fiber, precursors of vitamin A (29 mg carotenoids) (Rodríguez-Felix & Cantwell, 1988), and pectic substances that present a prebiotic and hypocholesterolemic effect (Guevara-Arauz & Ornelas, 2013). These chemical compounds can be beneficial to animal health.

There are some ways to increase the protein content in cactus plants including the use of fertilizers based on N and P, cloning and/or genetic modification (Gregory & Felker, 1992; Table 37.2), and solid fermentation by inoculation of prickly pear roots with nitrogen-fixing bacteria released as *Azospirillum* sp. (Caballero-Mellado, 1990). Fertilization based on the application of nitrogenous compounds can be an alternative to increase the content of crude protein. Gonzalez (1989) reported that crude protein content in fertilized cactus was close to double. The use of solid fermentation on nopal with *Aspergillus niger* developed an increase up to 12.8% of crude protein content (Oliveira, 2001), while the use of *Sacharomyces cerevisiae* reported an increase of 26% crude protein (Araújo et al., 2005). The use of acid hydrolysis (Foccher et al., 1991), and thermal pre-treatment are considered to improve the fermentation process.

Rodríguez et al. (2015) studied solid state fermentation using *Phanerochaete chrysosporium* (30 °C for 7 days at 180 rpm, fungal hydrolysis) followed by *S. cerevisiae* fermentation (37 °C for 48 h at 180 rpm), and achieved an increase in protein content from 0.042% to 26% (dry base). In addition, fungal hydrolysis generated an increase in free glucose from 8.32% to 18.9% (dry base).

Around the world (Table 37.3), as well as in Mexico, due to the need to feed cattle in the arid areas, since nineteenth century *Opuntia* has been used as fodder for livestock, mainly where the dry seasons are very long (Flores & Aguirre, 1979). In Monterrey and Nuevo León, two cities in the north of the country, farmers used to use 600 tons/day *Opuntia* to feed cattle. In Saltillo and Coahuila, 100 tons/day were used for this purpose (Granados & Castañeda, 1997).

**Table 37.2** Alternatives to increase the protein content in cactus

Description	Increase up to (%)	References
Using fertilizers based on N and P	12	Gonzalez (1989)
Genetic selection	11	Felker (2003)
Inoculation with N <sub>2</sub> -fixing bacteria	9.78	Felker (2003), Mascarua-Esparza et al. (1988)
Solid fermentation	12.8	Oliveira (2001)
	26	Araújo et al. (2005), Rodríguez et al. (2015)

**Table 37.3** Use of cactus as fodder on meat, milk and dual purpose production

Production	Livestock	Country	References
Meat	Cattle	Mexico	Flores and Aguirre (1979), Granados and Castañeda (1997), Lopez García et al. (2003)
		South Africa	De Kock (2003)
		EU	Felker (2003)
		Brazil	Cordeiro and Gonzaga (2003)
		Argentina	Ochoa and Barberab (2017)
		Ethiopia	
	Sheep	Mexico	Terblanche et al. (1971), Lopez García et al. (2003)
		UE	Griffiths (1905), Felker (2003)
		Chile	Riveros et al. (1990)
		WANA	Ben Salem et al. (1996), Nefzaoui et al. (1993)
		South Africa	De Kock (2003)
		Tunisia	Nefzaoui et al. (1993)
		Ethiopia	Flachowsky and Yami (1985)
	Goats	Mexico	Lopez García et al. (2003)
		Argentina	Ochoa and Barberab (2017), Guevara and Estévez (2003)
		Ethiopia	
	Rabbits	Brazil	Lukefahr and Cheeke (1990)
	Pigs	UE	Griffiths (1905)
	Oxen	UE	Griffiths (1905)
	Rams	Ethiopia	Flachowsky and Yami (1985)
Milk	Cattle	Mexico	Lopez García et al. (2003)
		Brazil	Santana et al. (1972), Lima et al. (1985), Santos et al. (1990), Lima et al. (1985)
	Goats	Chile	Azócar and Rojo (1991)
		Ethiopia	Azócar and Rojo (1991)
Dual purpose	Cattle	Mexico	González et al. (1998)
	Sheep	Mexico	González et al. (1998)
	Goats	Mexico	González et al. (1998)

The use of prickly pear as forage for cattle, sheep and goats has a significant impact by improving daily weight gain (0.1–0.6 kg/day). Supplementation with *Opuntia* increases not only milk production but also improves butter quality in terms of consistency and shelf life, as well as imparting an attractive “golden” color to the final product (González et al., 1998). Feeding sheep with *Opuntia* for to 525 days without drinking water is a resource to save animals from starvation without showing serious side effects (Terblanche et al., 1971). On the other hand, when *Opuntia* combined with corn, oats, and sorghum, it has a positive impact on the increase in weight of sheep and goats.

In Brazil, the use of *Opuntia* as fodder dates from the twentieth century (1915) (Pessoa, 1967); on 1932, the government established a great quantity of batch for

propagation of *Opuntia* in the semi-arid northeast region of Brazil due to the drought that year (Duque, 1973). The area planted with *Opuntia* in northeast Brazil is currently estimated to over 500,000 ha. Livestock farming in this region has a dual purpose: milk and meat (cattle, sheep, and goats).

Due to nutritional deficiencies of *Opuntia* in protein (but rich in soluble carbohydrates), it is not possible its use as single feed. Therefore, Brazilian farmers integrate it with corn or silage sorghum as an integral part on livestock production systems (Melo et al., 1992; Viana et al., 1966). Santana et al. (1972), and Santos et al. (1990) showed that using *Opuntia* cv. Gigante as single forage or in mixtures greater than 73%, Holstein cows lose weight. In lactating cows, milk production and live weight gain does not show difference compared with those fed with corn silage (Santana et al., 1972) or sorghum silage (Lima et al., 1985). Feeding rabbits with *Opuntia Palma redonda* (Brazilian var.) was attractive and immoderately increased the consumption (Lukefahr & Cheeke, 1990).

In the United States of America, the use of wild varieties of *Opuntia* previously “scorched” for the feeding of oxen, dairy and fattening cattle, as well as sheep and even pigs was described by Griffiths (1905). *O. ellisiana* presented the highest efficiency in the use of water (162 kg per kg of dry matter) and water accumulation of 170 tons per ha (in Texas). By supplying 45 kg of *O. ellisiana* per animal, it is possible to satisfy the water requirements for a period of 11.8 years (4315 days).

Spineless varieties have been generated with disadvantage that they are consumed by wildlife (Felker, 2003). In semi-arid areas of Santiago de Chile, the use of *Opuntia cladodes* (25%) combined with alfalfa as forage for sheep decreased water consumption up to 30% compared with those fed only with alfalfa (Riveros et al., 1990). In lactating goats feeding with diets based on *Opuntia cladodes* and alfalfa, milk production increases up to 55.4% (Azócar & Rojo, 1991).

The West Asia-North Africa region (WANA) comprises large areas with rainy winters and hot, dry summers. *Opuntia* was introduced to WANA region from Spain “moros”. However, large formal plantations were not established until the 1900s (Le Houérou, 1992). Currently, the combination of spineless prickly pear (*O. ficus-indica* var. *inermis*) with cereal straw is a nutritionally satisfactory solution to keep small ruminants in arid areas of WANA region. In WANA, prickly pear is used to supplement low-quality forages such as straw supplied to sheep, managing to increase the consumption of straw in proportion to the increase in prickly pear (Ben Salem et al., 1996). In straw treated with urea or ammonia, supplementation with prickly pear up to 55% in dry basis did not develop collateral digestive disorders and provides soluble fiber necessary for better use of non-protein nitrogen in the rumen. In addition, it was achieved a digestibility of 70% of the crude protein, and up to 50% crude fiber (Nefzaoui et al., 1993). *Opuntia ficus-indica* was introduced to Ethiopia from Mexico in the late nineteenth century, and is widely distributed in the semi-arid and arid regions, covering an area of around 30,520 ha, of which 48.6% is found in wild way and 51. Four percent are cultivated.

Since nineteenth century, the nopal plantation is common and extensive. Prickly pear has been consumed by almost all domestic animals, while livestock is totally dependent on *Opuntia* during the dry season. The government support the

development of *Opuntia* through two main organizations: Tigray Aid Society and the Regional Bureau of Conservation and Development of Natural Resources through which have promoted the selection, production and distribution of prickly pear varieties, the identification of diseases and the design of erosion control measures as part of its strategies (CFDP, 1994).

In South Africa, the nopal was introduced in the year 1911, and its cultivation has registered increases in production of 200–300% after the moderate application of nitrogen and phosphorous (Le Houérou, 1992; De Kock, 1980). Watering *Opuntia* without thorns is more efficient than irrigating a small area of alfalfa and generates almost twice as much forage production (De Kock & Aucamp, 1970). Cows and Sheep are fed during droughts using a silage prepared from crushed cladodes with straw in 84:16 ratio, and supplemented with molasses. Another alternative for silage, is to use *Opuntia* fruit and its cladodes ensiled with low-quality hay and supplemented with sources of protein (cottonseed, sunflower or urea), and minerals such as phosphorus and sodium (bone or salt) (De Kock, 2003). This can support dairy production in arid and semi-arid rural areas of South Africa. The most suitable supplement for feeding cladodes in South Africa is Karoo (a bush with a high protein content) or alfalfa (De Kock, 2003).

### 3 Biological Fencing/Vegetative Barriers

The use of various cactus species (*Opuntia ficus-indica*) as fence was described initially for Fernández de Oviedo (1526) in his work entitled “Summary of the Natural History of the Indies”. This concept was supported by Benavente (1541), and López de Gómara (1552), who’s often highlighting the use of cactus species as barriers or fences. Recently, in addition to their utilization as living fence to protect family household in dry regions, the nopal is used for erosion control of arid and semi-arid lands (Mondragon Jacobo & Chessa, 2013; Table 37.4).

In different parts of the world, the cactus is used as a fence. The fence plants in dry seasons are also used as forage while on summer for fruit production (Huffpost Algeria, 2015). In South Africa, cactus pear was planted as living fence and for its delightful fruit all around the arid and semiarid lands (Beinart & Wotshela, 2011). *Opuntia* spp. is also grown in Lebanon as natural fence and to produce “arak” (distilled alcoholic drink), which is characterized by its unsweetened anise-flavor. In the coastal and internal areas of Lebanon, cactus pear was initially introduced, it is destined mainly for fruit production (Chalak et al., 2012).

Sicily (Italy) is another example were cactus pear has been domestically grown since the eighteenth century. Its atypical appreciation of this plant is due to its multiple uses including fences in farming systems and emergency fodder (Barbera et al., 1991). Throughout North Africa and in parts of Italy and Spain, Torny cacti *O. ficus-indica* var. *amyclaea* (Ten.) A. Berger, var. *elongata* Shelle, and *O. violacea* Engelen var. *santa-rita* (Griffiths & Hare) L. D. Benson are used as defensive hedge for protection of gardens, orchards and olive groves. These hedges demarcate

**Table 37.4** Nopal as fence

Application	Country	References
Protect and delimit grounds	Mexico	Mondragon Jacobo and Chessa (2013), Guevara-Arauz (2020) <sup>a</sup>
Fence	Mexico	Guevara-Arauz (2020) <sup>a</sup>
	South Africa	Beinart and Wotshela (2011)
	Lebanon	Chalak et al. (2012)
	Italy	Paterson et al. (2011)
	Australia	Rao et al. (1971)
	Kenya	Hosking et al. (1988)
	Nambia	Hosking et al. (1988)
	Indonesia	Chinnock (2015)
	Zimbabwe	Walters et al. (2011)
Protection of cultivars	North Africa	Griffiths (1915)
	Italy	Griffiths (1915)
	Spain	Griffiths (1915)

<sup>a</sup>Personal communication

boundaries, so they make an excellent fence while helping to control erosion (Griffiths, 1915).

*A. subulata* (Muehlenpf.), *O. elatior* P. Miller and *C. fulgida* (Engelm) F.M. Knuth var. *fulgida* are utilized as a living fence in Australia, Kenya, Nambia, South Africa, Spain, Indonesia and Zimbabwe (Paterson et al., 2011; Rao et al., 1971; Hosking et al., 1988; Chinnock, 2015; Walters et al., 2011). Some of the advantages to use nopal (*Opuntia* spp.) for fencing are; (a) an excellent security for crops and homes, (b) a living fence provides, and (c) restore eroded lands. Le Houérou (1989) reported that the cost of establishing a fence using nopal is less than US\$ 60/ha, while a metallic fence (four strands of barbed wire) cost about US\$ 150/ha in Tunisia. The only disadvantage is the time necessary to be established for at least 2 years before it begins to function. There are alternatives to develop a fence, it is necessary to use the thickest pads from a plant, cut it and allow the scar to heal for about a week, and then plant the pads at 1.3–1.5 m spacing (White Dove Farm, 2015). Hedges and Fences of cactus can be developed by planting stems around 20–30 cm apart. The plants will grow together to form a wall of freely branching plants (3–6 m high). If the nopal is watered constantly, it can grow quickly so a fence can be established in as little as a 1 year. It is important to consider that spiny varieties of cactus pear or stems makes a good fence if it is compared with spineless varieties (University of California Cooperative Extension, 1989).

In landscape organization in countries or regions where no land registry exists, the nopal fences play an important role. In México the land-owners get together in “ejidos” where they agree if cactus hedges are often planted as testimony of land ownership and its register is set in the ejidal book. Additionally, nopal hedges have impact at local socio-economy in order to defending land rights and land ownership. In Tunisia planting of cactus fences on communal lands has a strong motivation and

popularity due to tradition dictates that tribal land may become the property of whoever among the rightful user has established a permanent crop on it.

## 4 Erosion Control

Galo et al. (1985) conducted a study where re-vegetation practices on 94 plantations were assessed and they established a correlation between environmental conditions and plantation characteristics. The best response of *Opuntia* in terms of establishment were found in steppe dry climate for 1 year old sites, likewise plantations in very dry climates had higher forage yields. This effect was attributed to set of initial conditions of the plantations, particularly precipitation. López et al. (1978) showed that a combination of rest, selective removal of undesirable shrub, and protection of *O. cantabrigiensis* or *O. engelmannii* was efficient for increasing nopal biomass yield, perennial grasses and solid organic matter content. The authors reported a 76% establishment for above species of nopal. *O. engelmannii* produced the greatest biomass yield, as it has more succulent pads with few spines. Other study carried out with *O. rastrera* pointed out that the best establishment and the highest green aboveground production occurred on deep clayed load soils with slope no greater than 2% (Maldonado & Zapien, 1977).

Among advantages of using prickly pear plantations with the aim of controlling land erosion in arid and semi-arid regions are: climatic stability, increase carbon capture, decreases the global warming, increased levels of soil fertility, stabilization of animal production and reduction of water needs of cattle (Table 37.5). However, these factors in many cases have not been evaluated, and therefore *Opuntia* plantations in various regions of the world have been economically underestimated. The

**Table 37.5** Advantage of using nopal as soil erosion control agent

Advantage	Source
<b>Direct</b>	
Improving soil physical properties	Monjauze and Le Houérou (1965), Bariagabre et al. (2016)
Increased levels of soil fertility	Le Houérou (1994, 1996)
Regulation of surface hydrological process	Vásquez et al. (2010)
Reduction of runoff and soil loss	Vásquez et al. (2010)
Climatic stability	Le Houérou (1994, 1996)
Carbon capture	
Decreasing the global warming	
<b>Indirect</b>	
Deforestation control	Vásquez-Alvarado et al. (2011)
Stabilization of animal production	Le Houérou (1994, 1996)
Reduction of water needs of cattle	Le Houérou (1994, 1996)

use of cactus hedges around badlands, arid lands and stony/rocky slope have been rehabilitated in efficiently, easy, and cheap way in Tunisia and Algeria (Le Houérou, 1994, 1996).

It is important to note that in the mid-twentieth century in North Africa, diverse strategies have been implemented to reduce water and wind erosion of grasslands; reverse desertification and restore vegetation cover in arid areas, slow and direct the movement of the dunes, improve the restoration of vegetative cover and prevent erosion in those areas through the planting of prickly pear (*O. ficus-indica* f. *Inermis*). Wide extensions of land have been planted with *Opuntia* in Algeria, Morocco and Tunisia. It is estimated that there are 1 million ha planted with two objectives: to combat erosion and desertification of the land and to provide fodder for livestock (Nefzaoui et al., 1993).

Cactus hedges established along contours play a major role in erosion control and land-slope partitioning. Soil physical properties and organic matter content under hedges and in adjacent areas are improved. The permeability and water storage capacity of aggregates in the topsoil increase, they are more stable, less sensitive to surface crusting runoff and erosion (Monjauze & Le Houérou, 1965). Bariagabre et al. (2016) reported that soils where *Opuntia* was planted showed high levels of soil organic carbon, soil total nitrogen, soil available phosphorus, soil bulk density, soil moisture, and electric conductivity compared to adjacent open areas.

The use of vegetation patches of prickly pear in semiarid lands from central Mexico, reduced by 98% runoff, and 99% soil loss. These results indicate a positive effect of *Opuntia* patches on the regulation of surface hydrological process (Vásquez et al., 2010). The cultivation of prickly pear in arid and semi-arid lands of Mexico generated a control in soil erosion and deforestation in various regions of the country, achieving 82% of reforestation and an erosion level considered stable of 0.4 ton ha<sup>-1</sup> year<sup>-1</sup> (Vázquez-Alvarado et al., 2011). One of the key factors to improve soils is water infiltration. Cactus cladode extract has been assessed and showed that they have the capability to improve water infiltration in soils (Sáenz et al., 2004).

## 5 Water Treatment

A common practice in some countries that farmers use cactus mucilage to purify drink water (Sáenz et al., 2004). The use of nopal is an excellent alternative to reduce the use of chemical products in the treatment process of industrial wastewater (Table 37.6). The general processes use alum as coagulant, polyacrylamide (PAM) as flocculant and lime as coagulant aid. In the case of potable water treatment, aluminium sulphate and ferric chloride are used as conventional coagulants. All these chemicals can be replaced by polysaccharides obtained from nopal (Saleem & Bachmann, 2019). Approximately 10% of Latin America population, principally low-income communities, remains without access to clean water. In response to this problem, a diverse water treatment methods including bacterial

**Table 37.6** Nopal as water treatment agent

Contaminant	Active component	Efficiency (%)	References
Pb <sup>2+</sup>	Nopal biomass	>94	Saleem and Bachmann (2019)
Suspended solids	Cactus juice	83.3–88.7	Sellami et al. (2014)
Suspended solids	Cactus juice + lime	>90	Sellami et al. (2014)
Kaolin, arsenic, and bacteria	<i>O. ficus-indica</i> mucilage	>90	Buttice and Alcantar (2014)
Turbidity	Cladodes	98.7	Nharingo and Moyo (2016)
Heavy metals	Fruit and peels mucilage	100	Saleem and Bachmann (2019)
Bacteria ( <i>E. coli</i> )	Nanofibrous membrane	100	Thomas et al. (2013)

by-products and plant-based materials have been assessed. *Opuntia ficus-indica* cactus has great potential because of its abundance, location, fast growth, and low cost.

In contaminated water, nopal (*Opuntia streptacantha*) biomass without any chemical or physical pretreatment, showed a significant Pb<sup>2+</sup> removal. The maximum adsorption capacity was 0.14 mmol g<sup>-1</sup> with an efficiency higher than 94% (pH 5.0 and 2.5 g L<sup>-1</sup> nopal biomass). The technological implication of these results is the development of an effective and economic technology to remove Pb<sup>2+</sup> from contaminated water (Miretzky et al., 2008).

Sellami et al. (2014) assessed the cactus juice compared with polyacrylamide as flocculant agent and found that depending on the wastewater's origin, the cactus juice computed removal efficiencies of 83.3–88.7% for suspended solids, and 59.1–69.1% for chemical oxygen demand. In addition, the use of cactus juice + lime enhanced the coagulation-flocculation process with efficiencies greater than 90% for both suspended solids and chemical oxygen demand.

The use of nopal mucilage as flocculant agent for kaolin, arsenic, and bacteria was assessed by Buttice and Alcantar (2014), who demonstrated that mucilage from *O. ficus-indica* increases settling rates of kaolin (50 g L<sup>-1</sup>) up to 12 times faster than control. So, it is a viable method for the aggregation and removal suspended acid-washed kaolin. Its efficiency combined with its abundance, low cost and accessibility become successful method for removal or reduction of suspended contaminants such as kaolin, arsenic, and bacteria.

The use of cladodes, fruit and peels mucilage and electrolytes showed maximum biosorption capacities up to 1000 mg g<sup>-1</sup> for dyes and up to 2251.5 mg g<sup>-1</sup> for metallic species. In addition, removal percentage computed was 98.7%, 93.6% and 100% for turbidity, chemical oxygen demand and heavy metals, respectively by coagulation-flocculation process. Polysaccharides from *Opuntia* possess multifunctional groups involved in wastewater remediation. The mechanisms associated to *Opuntia ficus-indica* flocculation-coagulation were charge neutralization and bridging effect (Nharingo & Moyo, 2016; Saleem & Bachmann, 2019). Chung-Yang



(2010) reported nopal as coagulant function by means of adsorption mechanism followed by charge neutralization or polymeric bridging effect.

Thomas et al. (2013) development a nanofibrous membrane (biodegradable water filter) based on mucilage of *Opuntia ficus-indica* and an organic polymer (chitosan, polyethylene glycol, poly lactic acid, poly vinyl alcohol) by electrospinning, which can be used in filtering contaminants from water. This invention provides sustainable technologies, environmentally friendly, non-toxic, and biodegradable methods of water treatment that are economically competitive and affordable. The electrospun nano-fibrous membrane remove bacteria (*E. coli*) from water and possess several attributes that make them very attractive in water filtration technology, these include but are not limited to, high porosity, pore sizes ranging from tens of nanometers to several micrometers, interconnected open pore structure, and large surface area per unit volume. In Mexico, the nopal juice is being researched with aim to clean wastewater generated from subway tires wash.

## 6 Additive Used in Construction

In construction industry recent interest has been shown in the nopal mucilage due to diversity of applications and properties as adhesive, anti-corrosive, and as additive for paints and adobe (Table 37.7). In Mexico, with a long history of using, the cactus mucilage has been applied in combination with lime as plaster on adobe or bricks walls, as well as a water barrier in stucco (Sáenz, 2013; Torres-Acosta, 2007; Torres-Acosta & Martínez-Madrid, 2005). Cladode juice was traditionally added to lime, as a practice that traced back to antiquity, as an organic adhesive to restore and protect historical buildings (Cárdenas et al., 1998). The authors assessed the addition of cactus juice up to 1.95% (extracted by boiling cladodes) to lime. The addition of cactus juice developed a drastic reduction in maximum stress and in the rate of deformation compared with control. The mechanical properties increased owing

**Table 37.7** Functional properties of nopal applied in construction

Properties	Application	References
Adhesive	Mud blocks, mortars and concretes	Guillen et al. (2019), Ramírez-Arellanes et al. (2012), Pérez-Castellanos (2009)
Plaster	Adobe or bricks walls	Sáenz (2013), Torres-Acosta (2007),
Water barrier	In stucco	Ramírez-Arellanes et al. (2012), Torres-Acosta and Diaz-Cruz (2020)
Improve durability	Lime-based mortars and concretes	Torres-Acosta et al. (2005), Ramírez-Arellanes et al. (2012)
Anti-corrosive	Reinforcing steel in concrete	Pérez-Castellanos (2009), Dúran-Herrera et al. (2012), Lopéz-León et al. (2019), Martínez-Molina et al. (2016)
Resistance to erosion	Adobe bricks	Martinez-Camacho et al. (2008)

to the formation a homogeneous network in which cactus pear mucilage penetrated the calcium hydroxide without modifying the structure.

Natural polymers have been used since ancient times to improve durability of lime-based mortars and concretes. In Mexico, prickly pear mucilage has historically been used as an additive for lime mortars because it prevents rapid drying of the mortar, helping to retain the moisture it requires to set properly without cracking. Chandra et al. (1998) assessed cactus extracts in a Portland cement mortar and they found that cactus extracts increase the plasticity, improve water absorption and freeze-salt resistance of the mortar. The components of the cactus extracts (polysaccharides and proteins) interacts with calcium hydroxide produced during hydration of Portland cement to conform complexes affecting the crystallization process (Peschard et al., 2004).

Ramsey (1999) studied cladode mucilage (10%) as stabilizer for adobe construction blocks and found a lightly advantage compared them with the lime traditionally used. Probably, this result was due to the low dosage and the method used to prepare cactus mucilage as stabilizer (washing and soaking the cladodes in water for 18 days). In a similar work, Cárdenas et al. (1997) assessed the use of cladode juice in lime pastes and stated that the juice weakened the texture of lime paste.

In a study by Hernández and Serrano (2003) on the addition of lyophilized mucilage cladodes (0.5 g) to mortars (plaster, silicate sand and lyophilized cladode mucilage), they found a better compression resistance than the control mortars. Torres-Acosta et al. (2005) and Ramírez-Arellanes et al. (2012) stated that addition of cactus mucilage to cement mixes did improve the durability of the cement products. The adhesive properties of lime are enhanced, and water repellence is improved. Martínez-Camacho et al. (2008) treated adobe bricks with nopal previous immersion in alcohol-water solution, this treatment increased their resistance to erosion. The structural properties of treated material showed that nopal coats the adobe small particles, generating with this a homogeneous erosion and not selective and this impregnated material is stable up to 200 °C. This treatment aims to restore a building with cultural and historical background “*Nuestra Señora del Pilar mission*”.

The addition of mucilage in lime and marble mortars improves consistency, adhesiveness, shrinkage percentage, required stirring power and supported load. The best results were obtained with marble: lime ratios of 1.5:1 and mucilage: water of 2:1. Due to its good suspension of solids, acceptable fluidity for injection, high adhesiveness and high compressive stress. This mixture represents a flexible lime and marble mortar for the consolidation of mural painting by the injection method (Pérez-Castellanos, 2009).

Mucilage from nopal is also applied in the cement industry due to the advantages it generates including reduce retraction, corrosion inhibitor, viscosity modifying agent, reduces deformity of the paste, maintaining fluidity and maintaining viscosity, it is an air entrainer, holds water longer (therefore, hydrates it longer) which aids in remote transportation (Pérez-Castellanos, 2009; Dúran-Herrera et al., 2012). Barajas et al. (2009) studied nopal gum “slobber” as a consolidant to preservation monolithic pieces through impregnation of the mineral with nopal. The use of this organic material cover the mineral particles with a smooth layer, these solutions

react and lead to microporosity. León-Martínez et al. (2014) proposed the use of two new bio-polymers: nopal mucilage and marine brown alga extracts as alternative viscosity-enhancing admixtures for the production of stable, cohesive, and homogeneous pastes, mortars and self-consolidating concrete (cement-based materials). Nopal and brown alga extracts dispersions showed a shear-thinning behavior which produce a significant increase on the share viscosity and yield-stress in pastes, mortars and self-consolidating concrete, which increased with increasing brown alga extract concentration and this depend on their molecular nature.

Díaz-Blanco et al. (2019) based on the result obtained by Dúran-Herrera et al. (2012) stated that nopal mucilage can delay the corrosion of reinforcing steel in concrete. This natural additive at concentration 1:3 was able to delay the onset of corrosion and protect the reinforcing steel with an efficient of 86%, acting as a retardant of the setting of the concrete, as a result of maintain a corrosion rate between negligible and low throughout the test period. They also affirmed that nopal mucilage within the concrete matrix maintains the ideal conditions for the steel to acquire a state of passivation.

Lopéz-León et al. (2019) evaluated the mucilage from *Opuntia strpetacantha* (0.5 g) as a corrosion-resistant hybrid coating of rebars through immersion for 24 h in a 3.5 wt% NaCl solution. The mucilage hybrid coating enhanced corrosion protection due to the homogeneity of the mucilage in the coating. Thus, it has favorable properties as a barrier due to its ability to obstruct the diffusion of aggressive species by trapping them in a coating structure, which avoid their adsorption on the metallic surface, acting as a corrosion inhibitor due to its semipermeable behavior, where only water molecules flow through its pores.

A clear example about the benefices of nopal in construction is the development of a mud block using ecological materials like soil mixtures, cellulose (recycled paper), and *Opuntia ficus* extract (mucilage) as a binder. The soil and the wastepaper are grinding down to a particle size smaller than 3 mm, the mixture is hydrated, and its pH was adjusted (<8) trough addition of lime. Follow by a stabilization that contributed to the cementation of particles and the subsequent addition of mucilage; the components are homogenized until moldable pasta is obtained. This is emptied into molds and left to dry for 9 days. The mud blocks showed a compressive strength higher 76 kg/cm<sup>2</sup> than non-structural conventional bricks 60 kg/cm<sup>2</sup>, and structural conventional bricks 70 kg/cm<sup>2</sup>. In addition, not only moisture absorption is reduced by up to 30%, but also the organic brick weight up to 25% were computed. Moreover, at a 10% lower cost since no firing is required without generating CO<sub>2</sub>, the production times are shorter, mitigation of climate change, fewer worker diseases and mitigation of climate change. So, this kind of material promotes the conservation of natural resources and reduce the environmental impact (Guillen et al., 2019).

A developed research by Torres-Acosta and Diaz-Cruz (2020) showed that nopal derivatives (exudate nopal mucilage and cooked nopal mucilage) may act as clogging sponge-like biopolymer within the cement matrix pores, stopping water and chloride transport into concrete. Exudate nopal mucilage exhibited improved durability index values up to 20% (total voids percentage/effective porosity decreases and saturated electrical resistivity/compressive strength increase), and rapid

chloride permeability index was improved up to 30%. For its part, cooked nopal mucilage produced superior improvements to the control mixture between 20% and 40%. On the other hand, addition of dehydrated nopal powder did not improve substantially concrete durability performance, except chloride transport: additions <2% decreased rapid chloride permeability index value up to 10%.

## 7 Improving House Paint and General Paints

The mucilage's from nopal had a traditional use for improving house paint in some countries (Sáenz et al., 2004). Studies are under way on the use of cladode extracts as an additive which function as adhesives in paints (Sáenz, 2013). In Mexico, small pieces of nopal pads are mixed with lime solutions to improve the quality when this solution are used to pain walls or fences. In this process, mucilage give fixing properties. Other example is "*Ecopal*" a multipurpose paint made form slime of prickly cactus, limonene solvent and polystyrene, it is friendly with environment and also works for waterproofing (Vargas, 2015).

## 8 Nopal Mucilage in Edible Coatings and Packaging Applications

By definition edible films and coatings are primary packaging materials, they are able to preserve foodstuffs and extend their shelf life, based on polymeric matrixes directly applies on the foodstuffs surface or between constituents by immersion, spraying, electro-spraying, which is followed by drying. Generally, these films and coatings are developed from biopolymers and enriched with different kind of additives as essential oils, plant extracts, enzymes and probiotics providing biological and functional properties such as antioxidants and antimicrobial activities. The advantage of biopolymer-based material over polyolephine-based materials are their sustainability and safer for human health (Gheribi & Khaoula, 2019).

Nowadays, edible coatings and films have been projected as operative solution to prevent physical and nutritional quality foods losses. This is no new, cellulosic and waxy coatings were used in the mild-twentieth century to reduce weight loss and develop the shine and brilliance of fruit and vegetables (Hassan et al., 2018). The advantage of use edible coating in horticultural products and perishable food stuffs is the coatings acts as a modified atmosphere system or packaging in which the metabolism is reduced as a result to the change in the micro-environment generated between the product tissue and the thin layer coating development high CO<sub>2</sub> and H<sub>2</sub>O vapor levels and low O<sub>2</sub> levels. This reduction in metabolism, also decreases the respiration rate and deteriorative reactions like browning after like reflection on surface or physical damage generated by instability of phenolic compounds or

enzymatic activity such as oxidase and peroxidase. Coatings acts like a selective barrier to gases and water transfer by slowing foodstuff dehydration maintaining its turgor. The change in atmosphere (high CO<sub>2</sub> levels, and low O<sub>2</sub> levels) decrease the growth of spoilage and pathogenic microorganism without affecting color, taste or smell of the coated product (Guevara-Arauz, 2010).

Cactus mucilage is consider a trendy versatile biopolymer, eco-friendly, available, and profitable alternative to petroleum-based materials (Valdés & Garrigós, 2016). Also, this biopolymer is one of the most abundant carbohydrate in cactus plant and it is consider a valuable raw material for added-value biomolecules with various industrial applications (Ochoa & Barberab, 2017). This polysaccharide has been used as polymeric matrix and reinforcing or blending agent for development of bio-composite materials and as a green material that could be applied in biomedical, construction, furniture and packaging industries (Kumar et al., 2017, 2018). The mucilage content in cladodes is higher in older cladodes and increases as a response to drought in order to preserve the plant. It is more abundant in cactus cladodes (19–24% dry weight) than in fruit peels (4.1%), fruit pulp (3.8%) and flowers (18.3%) (Habibi et al., 2004; Matsuhira et al., 2006; Ammar et al., 2015).

Cactus mucilage is a branched heteropolysaccharide (about 33–55 sugar residues) with high molecular weight conformed mainly of arabinose, galactose, xylose, and rhamnose with slight variations in the content. It has the ability to swell when dissolved in water and to form colloidal and viscous suspensions (Sepúlveda et al., 2007). Consider as hydrocolloid, *Opuntia ficus-indica* mucilage has great water-holding capacity, which permit to the plant growing under water-stress conditions, playing an important role in the physiology of the plant (Sáenz et al., 2004). Mucilage extraction yields depend on the plant organ, the cactus species, and the extraction method. Furthermore, the extraction parameters deeply influence the extraction yield (Sepúlveda et al., 2007). Due to its properties, cactus mucilage can be used as a reinforcement agent in polymeric matrices, to develop edible films and coatings as well as to form bio-composites when it is blended with other polymers and it can be consider eco-friendly material (Del-Valle et al., 2005; Gheribi et al., 2019a, b; Lopez-Garcia et al., 2017; Guadarrama-Lezama et al., 2018).

### 8.1 *Cactus Mucilage as a Coating Material*

Cactus mucilage has been effectively used as a coating material for highly perishable horticultural and minimally processed products (fresh cut or sliced ones; Table 37.8). Del-Valle et al. (2005) used cactus mucilage for first time on strawberries. This treatment showed better firmness than control and enhanced resistance to mechanical damage during storage and, thereby, reduce economic losses. Furthermore, the color of coated strawberry was maintained for 5 days and their sensory attributes were preferred over uncoated ones during storage period.

Oluwaseun et al. (2014), used cactus mucilage-based coating on papaya fruits extend their shelf life and reported that ripening was delayed during storage at room

**Table 37.8** Application of nopal based edible films and coatings on horticultural products

Horticultural product	Total shelf life	Increase in shelf-life	References
	(days) <sup>a</sup>	(days) <sup>a</sup>	
Strawberries	14	5	Del-Valle et al. (2005)
	21	12	Ruiz-Hernández (2009)
Blackberries	10	6	Najera-García et al. (2018)
Papaya	21	7	Oluwaseun et al. (2014)
Pineapples	28	9	Trevino-Garza et al. (2017)
Sliced pineapple	6	5	Zambrano et al. (2017)
Mango	16	4	Girma et al. (2019)
Sliced mango	9	6	Alikhani (2014)
Kiwifruit slices	12	5	Allegra et al. (2016)
Figs	14	7	Allegra et al. (2017)
Guava	16	4	Zambrano et al. (2018)
Yam	10	6	Morais et al. (2019)
Tomatoes	21	13	Bernardino-Nicanor et al. (2018)
	30	19	Olicón-Hernández et al. (2020)
Loquat fruits	35	30	Kahramanoğlu (2020)
Fresh cut potatoes	5	5	Wu (2019)
Roasted peanuts	29.5	21	Mestrallet et al. (2009)

<sup>a</sup>Compared with control

temperature as a result of change in its internal atmosphere. Also, aerobic psychrotrophic and mesophilic bacteria counts decreased from 11 to 4–6 CFU/g and from 9 to 4–6 CFU/g, respectively. Similar results reported the efficacy of cactus mucilage coatings for reducing microbial growth (Trevino-Garza et al., 2017; Allegra et al., 2017).

For their part, Trevino-Garza et al. (2017) applied bio-composites materials of mucilage/chitosan coatings on fresh-cut pineapples and found that it is effective to protect the product and extending its shelf life by 6 days in comparison with control (uncoated ones). Coated fruit exhibited higher firmness than uncoated ones after 18 days of storage at 4 °C. The coatings act by decreasing water vapor transmission rate and weight loss by almost 10%. The results indicated that the coating delayed respiratory metabolism reactions. Besides, coating significantly reduce *Listeria monocytogenes* and *Salmonella typhi* counts. They also reported marked reduction on yeast and mold, total aerobic, and psychrotrophic counts for uncoated fruits compared with coated ones from 6.6 to 3–5 UFC/g; 4.7 to 3.6–4 UFC/g; 4.1 to 2.4–3.8 UFC/g respectively, at the end of storage of 4 °C. The authors did not take in consideration the modified atmosphere created by coating and attributed the reduction in microbial growth to antimicrobial effect of chitosan and low storage temperature (4 °C).

In another research, Allegra et al. (2017) showed that *Opuntia ficus-indica* mucilage-based coating applied in breba figs significantly lowered the growth of

*Enterobacteriaceae* compared with uncoated ones. However, the coating did not induced any microbial growth inhibition in other kind of microorganism throughout storage period at 4 °C. Zegbe et al. (2015) applied edible films to coat guava fruit and found that color, firmness, soluble solids, and dry matter concentrations were maintained throughout room temperature storage. But, the incorporation of plasticizers (glycerol and polyethylene glycol) in edible film formulation increased fruit weight loss. Mucilage from *Opuntia elatior* Mill used for coating guava fruits affected significantly firmness, pH, titratable total acidity, total soluble acids, and sensory attributes (Zambrano et al., 2018),

Tomatoes coated with mucilage from *Opuntia Robusta* maintained their firmness and showed a reduction on weight loss. However, lycopene content remained higher in uncoated tomatoes after 21 days of storage (Bernardino-Nicanor et al., 2018). Morais et al. (2019) applied a composite from cactus mucilage and cassava starch to minimally processed Yam and found that mucilage coated product showed lower weigh loss than roots coated with the composite mucilage-starch. They also found an increase in polyphenol content, likely synthesized as a defense mechanism against browning reactions occurring in minimally processed yam. In general, the use of nopal mucilage as a coating material reinforce firmness, brings better appearance, extended shelf life of cactus mucilage-coated products and preserving their quality attributes. Even though mucilage and partially Tween 20 addition increased yeast growth, their levels were still below the threshold for yeast spoilage at the end of storage period.

Allegra et al. (2016) packed kiwi fruit slices coated only with cactus mucilage or with mucilage-Tween 20, under passive atmosphere at 5 °C for 12 days. Slices treated only with mucilage retained the highest firmness and showed significant beneficial effects on the visual and flavor score of the kiwi-fruit slices until the end of the shelf life period. In addition, both treatments allowed to generate a significant higher firmness and lower weight loss than untreated slices, until 5 days of shelf life. Even though mucilage and partly tween 20 addition increased yeasts growth, their levels were still below the threshold for yeast spoilage at the end of the monitoring period.

Mangoes (*Mangifera indica*) coated with Aloe gel (50%) and cactus mucilage (75%) in combination and/or alone retard chemical and quality deterioration and keep good appearance up to 16 days of storage (Girma et al., 2019). Aloe gel had significant effect on all sensory attributes, while cactus only on color, appearance and over all acceptances. Alikhani (2014) treated sliced mangoes by coating with *Opuntia* mucilage and rosemary oil microencapsulated then were overwrapped using PVDC film and finally stored at 6 °C. This treatment inhibits the decay incidence and slowed microbial growth. In addition, the loss of ascorbic acid, changes in color, activity of peroxidase enzyme were retarded. The author conclude that the use of cactus mucilage effectively prolong the quality attributes and extend the storage life of sliced mango up to 9 days.

Ruiz-Hernández (2009) applied three different kind of coatings on strawberry: (a) “MAG” mucilage (4%), olive oil (1%) and glycerol (1%); (b) “MPG” mucilage (4%), polyethylene glycol (1%), and glycerol (1%); and (c) “C” chitosan (1%),

acetic acid (2.5%), and olive oil (0.6%). The coated product was packed in perforated glass polystyrene and stored at refrigeration throughout 21 days. The coats applied to strawberry kept the firmest and sensory characteristics. By its part MAG and C treatments kept the color for long time. In addition, the strawberry coated with a layer of the MAG formulation was the one with the least weight loss at the end of the storage period. The C treatment delayed mold and yeast growth. Besides, MAG films showed both the lowest water vapor permeability (2.02–4.21 g m<sup>-2</sup> h mmHg) and the O<sub>2</sub> permeability (13.1 mL m<sup>-2</sup> h<sup>-1</sup> Pa<sup>-1</sup>), while C films were more lightly, resistant and stronger despite to show the lowest thickness (34.2–48.9 µm) compared with mucilage formulated films (92.3–167.2 µm). The author conclude that mucilage coating applied to strawberry is a good treatment to keep shelf life.

Olicón-Hernández et al. (2020) designed an edible film based on cactus mucilage (20%), chitosan (2%), and glycerol (3%) as an alternative against fungi infections on horticultural products. This film showed a strong antifungal effect against *Rhizopus stolonifer* *in vitro* and *in situ* condition increasing the shelf life of tomatoes. The resulting film was homogenous, flexible, luminous, slightly dark and cumulative viscosity.

Zambrano et al. (2017) evaluated the effect of cactus mucilage (10% and 20%, w/v) as edible coating on cut pineapple physicochemical, sensory and quality parameters. Cactus mucilage helped to reduce the detrimental effect caused by minimal processing of fresh cut pineapple. The sliced pineapple (1 cm thick) were treated with sodium hypochlorite then immersed in a solution of mucilage for 1 min, finally were stored at 6 °C for 7 days. The coating was effective in retarding the weight loss and firmness. Thus, judges had preference coated samples at the end of storage for taste, color, texture and appearance attributes.

Kahramanoğlu (2020) assessed four different cactus mucilage based biomaterials on the postharvest life and storage quality of loquat (*Eriobotrya japonica* Lindl.) fruits var. 'Morphitiki'. The author found that cactus mucilage extract (CME), CME + *Nigella sativa* oil, CME + propolis extract, CME + cinnamon oil treatments were effective in maintaining the postharvest quality of loquat fruits by reducing weight loss, positively affecting fruit firmness, preventing fruit browning and reducing decay incidence. Besides, 0.5% *Nigella sativa* oil (Ns) or 0.5% propolis extract (PEx) treatments can be used to store loquat fruits with acceptable quality for up to 35 day at 4 °C.

Najera-García et al. (2018) mixed mucilage from *O. heliabravoana* Scheinvar and thermoplastic starch to produce an edible coating that applied to blackberries. After 10 days of storage, the coated fruits did not show significant different from those of the control on the physicochemical variables. Nevertheless, the microbial growth of coated blackberries was significantly lower than that of the uncoated fruit.

Mestrallet et al. (2009) applied *Opuntia ficus-indica* and algarrobo (*Prosopis* spp.) pod syrup coatings on roasted peanuts stored at 23 °C for 112 days. The product was more resistant to lipid oxidation and development of rancid flavor, so the stability of roasted peanuts was improve through preventing loss of their sensory and nutritional quality. The prickly pear coating showed higher antioxidant activity.



Therefore, its addition as coating provided protection against lipid oxidation. After 20.7 days of storage at 23 °C, peroxide value in roasted peanuts coated with prickly pear syrup reached 10 meq O<sub>2</sub>/kg, while in roasted peanuts without treatment this value was reached in only 8.5 days.

Aquino et al. (2009) applied a combination of cactus mucilage (35 mPa) blended with citric acid (1%), and sodium bisulphate (500 ppm) as coating to inhibit the browning process during the drying (50 °C and air velocity of 2 m s<sup>-1</sup>) of banana Roatán (*Musa Cavendish*) (5 mm thickness). The treatment applied to bananas formed an edible coating on the surface of sliced bananas that gives shine and diminished the browning of bananas during drying.

## 8.2 Cactus Mucilage as a Film Packaging Material

Biodegradable edible films made up from natural polysaccharides are trendy due to over accumulation of solid waste and their impact on environment. On the other hand, their potential industrial applications is as an excellent alternative to overcome the above problems. In the last decade, cactus mucilage from different plants and varieties has been used to develop stand-alone and composite bio-based films with unique properties. Between the outstanding raw materials are *Balangu*, cactus, chia, *Dracocephalum moldavica* seeds, flax, quince, and okra fruit. Being non-toxic and intended for human consumption are other advantages of these films. The main process to perform this kind of biodegradable based-mucilage film are based on thin polymeric layers formed by a dry (extrusion) or humid (casting) procedure (Gheribi et al., 2018; Jouki et al., 2014; Dick et al., 2015; Karami et al., 2019; Sadeghi-Varkani et al., 2018; Beigomi et al., 2018; Cotrim et al., 2016).

Espino-Díaz et al. (2010) studied the effect of calcium on developed cactus mucilage-based films using glycerol as plasticizer and found that between pH 4 and pH 8, films were elastic but strong enough to be characterized. The values of tensile strength (TS), elongation at break (EB) and water vapor permeability (WVP) for these mucilage films were 0.95 MPa, 24% and 98–147 g mm/m<sup>2</sup> day kPa,

**Table 37.9** Mechanical and barrier properties of mucilage based films

Tensile strength (TS; MPa)	Elongation break (EB; %)	Water vapor (g mm/m <sup>2</sup> day kPa)	References
0.95	24	98–147	Espino-Díaz et al. (2010)
>1	33	63.8	Gheribi et al. (2018)
0.5–2.7	NR	20.22–180.40	Lira-Vargas et al. (2014)
1.65	14	NR	Gheribi et al. (2019b)
30–50	10–70	0.852–3.066 × 10 <sup>12</sup>	Dominguez-Martinez et al. (2017)
4.44	NR	1.41	González et al. (2019)
0.25–0.36	NR	6.55	Salinas-Salazar et al. (2015)

NR not reported

respectively (Table 37.9). The films color varied from light yellow to yellow-green at low and high pH values, respectively. Chroma values were higher in mucilage films at high pH values without calcium. Color saturation was influenced by pH and calcium content.

Gheribi et al. (2018) assessed the effect of different plasticizers on edible films developed using cactus mucilage and reported that sorbitol-plasticized films showed the best TS and WVP, while polyethylene glycol (PEG) 400 plasticized films showed the highest glass transition temperature 49 °C and thermal stability up to 171 °C. In addition, glycerol plasticized films had TS > 1 MPa, EB 33%, and WVP 63.8 g mm/m<sup>2</sup> day kPa. Comparing, the films developed by Gheribi et al. (2018) had higher TS and EB values than those performed by Espino-Díaz et al. (2010). On the other hand, WVP value for Gheribi et al. films was lower than Espino-Díaz et al. films.

### 8.3 Development of Composite Materials Using Cactus Mucilage

Composite materials using cactus mucilage (0.5%), gelatin (0.25–0.5%), and beeswax (0.25–0.5%) plasticized with glycerol were produced by Lira-Vargas et al. (2014). The developed composites had intermediate to high roughness. The addition of beeswax increased the lumpiness and decreased the transparency of composite film. The ternary blend showed an increased TS (0.5–2.7 MPa), and decreased water vapor ( $13\text{--}116 \times 10^{-12}$  mol m/s m<sup>2</sup> Pa), O<sub>2</sub> ( $3\text{--}14 \times 10^{-12}$  mol m/s m<sup>2</sup> Pa), and CO<sub>2</sub> ( $3\text{--}9 \times 10^{-12}$  mol m/s m<sup>2</sup> Pa) permeability. Despite of improving the mechanical and barrier properties, the ternary composites characteristics are still poor, which limit their practical and industrial application.

Gheribi et al. (2019b) developed a composite conformed by cactus mucilage and polyvinyl alcohol (PVA). The optimal blend was the composite at 80:20 (mucilage:PVA). The authors showed that PVA inclusion increased TS (165%), EB (14%), and the water contact angle (24%), confirming an improvement on physical, mechanical, thermal and barrier properties of mucilage-PVA films. In another research, the properties of ternary composites made of chitosan, PVA, and cactus (*O. tormentosa*) mucilage (10%) were studied by Dominguez-Martinez et al. (2017). The inclusion of mucilage contributed to composites stability and homogeneity with lower TS values (30–50 MPa), EB values (10–70%) and higher WVP values (852–3066 mL/mm<sup>2</sup> day Pa) and water uptake than neat PVA and chitosan films.

Guadarrama-Lezama et al. (2018) developed a binary-composite made of citric pectin and cactus mucilage at different proportions and showed that films microstructure was compact, smooth and homogeneous even at high concentrations (>12%) with increased thermal stability and decreased water vapor permeability and solubility. Lopez-Garcia et al. (2017) assessed the chemical, thermal, and mechanical properties of composited films based on starch-chitosan-PVA-mucilage

*Opuntia joconostle* composite throughout two methods, the direct incorporation of mucilage and the addition of water ethanol extracted mucilage. The first treatment caused microphase separation in the film network, while the second treatment had no clear microphase separation (aggregation), this means that the film components were homogeneously dispersed in extracted mucilage and indirectly-added mucilage. Films developed adding directly mucilage showed mechanical properties slightly lower than films with extracted mucilage.

Cactus mucilage from *Cereus hildmannianus* fruits (widespread cactus in Brazil) was applied by Damas et al. (2017) to develop glycerol-plasticized edible films with interesting functional properties. Similarly, Gheribi et al. (2019b) used a prickly pear peel for the extraction of mucilage that further used to develop flexible and cohesive films with a smooth surface and good thermal stability.

Ayquipar-Cuellar et al. (2020) developed various films using agro-industrial wastes in specific prickly pear peel mucilage, potato husk starch, glycerine, and vinegar (acidifying) with the aim to be applied in horticultural products. All edible films showed good barrier properties and thereby very low permeability. In addition, cactus mucilage and glycerine contents led to films with higher thickness, opacity, moisture and water retention capacity, while potato starch content influences the percentage of water solubility.

An intelligent packaging was developed based on incorporation of betalain (0.25, 0.50 and 1.0 wt% on starch basis) from red pitaya (*Hylocereus polyrhizus*) peel extract in a starch/polyvinilo alcohol matrix. The use of betalain extract (1.0 wt%) in films allowed to monitor the freshness of shrimp, as a result of a visible color change due to the accumulation of volatile nitrogen compounds (ammonia) during the spoiling process as a result of color change under alkaline conditions. The extract addition improved, not only the compactness mechanical, antioxidant, and antimicrobial potential of the films, but also enhanced water vapor barrier and ultraviolet-visible light barrier ability (Qin et al., 2020).

Scognamiglio et al. (2020) blended cactus mucilage in gel form with a self-produced thermoplastic potato starch (TPS), and glycerol (30%). Three methods of extraction were compared bare maceration, mechanical blending, and mechanical blending plus maceration. The last process allowed to get the high extraction yield and the larger deformation of the samples with respect to the control. The authors also reported that the mechanical properties were not completely satisfactory since the effect of calcium and magnesium contained in the mucilage did not improve the rigidity of TPS films.

González et al. (2019) successfully fabricated edible films containing mucilage from different Nopal cultivars (Villanueva, Jalpa, and Copena F1). The edible films developed with Copena F1 showed the lower water vapor permeability ( $1.63 \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ ), high solubility (81.2%), and high resistance ( $4.44 \text{ N mm}^{-1}$ ). The characteristics in color, yellowness index, transparency (3.81); and light transmission rate (48.9%) allow their use for foods susceptible to light exposure, with better visual effect.

Due to importance related with cactus mucilage properties researches have explored the development of edible coatings and films with the main goal to apply

this to horticultural products. Salinas-Salazar et al. (2015) characterized films based on *Opuntia ficus-indica* mucilage (0.5% w/v), beeswax (0.5%, w/v), and gelatin (0.5%, w/v) and found that this kind of films had the lowest water vapor permeability ( $7.578 \times 10^{-11}$  g/m s Pa). Edible films with 1.5% (w/v) gelatin, and 0.5%, 1% and 1.5% (w/v) of mucilage had the best mechanical properties (0.25–0.36 MPa). Orozco (2017) developed and characterized films based on cactus mucilage (5%) + citric pectin (2%), and glycerol (5 mL). Ayquipa (2018) characterized films performed with prickly pear peel mucilage, potato peel glycerol, and vinegar. The author coincide on the fact that this sort of films may be useful for application in the preservation of fruit.

Overall, cactus mucilage films exhibited unique properties; however, barrier properties are inferior to polyoleophine-based films (conventional plastic materials), which limit their industrial application. Cactus mucilage is compatible with many biodegradable polymers such as PVA, chitosan, starch, and citric pectin, which may lead to countless industrial applications.

## 9 Nanoparticles and Nanocomposites Based on *Opuntia ficus-indica*

Recently, organic nanoparticles, as for example cellulose, pectin and mucilage, are being synthesized due to its importance in diverse areas such as in food and pharmaceutical industries, and in packaging technologies. Their more relevant characteristics are availability, functionality, easy synthesis, properties, low cost, high biodegradability, biocompatibility, and high strength. As a result, biopolymer nanofibers are being used as reinforcement material in electronic devices medicines, and many other products. However, the use of spherical nanoparticles has not widely been applied in the industry at present. It is important to highlight that dimensions of nanoparticles depend on numerous factors that include the source from the cellulose was obtained and the precise preparation conditions among others.

### 9.1 Cellulose Nanoparticles from *Opuntia spp.*

Malanine et al. (2005) obtained nanocomposite materials prepared from *Opuntia ficus-indica* cladodes and model amorphous matrix composed of an aqueous suspension of a copolymer of styrene and butyl acrylate. In combination, the highly reactive surface of the cellulosic filler which presenting a high density of hydroxyl groups, the resulting possibility of entanglements, and the very high aspect ratio of the filler, generated a high performance material.

Marin-Bustamante et al. (2017) used the waste generated from de-thorning process of nopal (40,000 tons/year) to generate a new biodegradable nanoparticles

**Table 37.10** Green synthesis of nanoparticles from or using cactus components

Source	Nanoparticle	Applications	References
Cladodes	Cellulose	Reinforcement material	Malanine et al. (2005)
Thorns	Cellulose	Reinforcement material	Marin-Bustamante et al. (2017)
Pectin	Ca <sub>10</sub> (PO <sub>4</sub> ) <sub>6</sub> (OH) <sub>2</sub>	Bone regenerating material	Gopi et al. (2015)
	CdS	Treatment of cancer cells	Kandasamy et al. (2019)
		Detection of cancers and other diseases	
		Anti-bacterial and fungal activities	
Extracts	Au/Li	Periodontal disease	Alvarez-Bayona et al. (2019)
	ZnFe <sub>2</sub> O <sub>4</sub>	Oxidation of glycerol to formic acid	Kombaiah et al. (2017)
	ZnO	Absorb ultraviolet light in sunscreen	Francisco-Escudero et al. (2017)
	Ag	Antimicrobial activity	Silva-de Hoyos et al. (2012), Ledezma et al. (2014), Baylon et al. (2015)

taking advantage of its huge amount of hemicellulose and cellulose. The size of developed cellulose nanoparticles were ranged from 24 to 122 nm. They concluded that cellulose nanoparticles from nopal thorn could be used as reinforcement material in various industries (Table 37.10).

## 9.2 Green Synthesis of Nanoparticles Using Cactus Pectin

Green chemistry throughout the use of biomolecules from plants, alga, fungi, bacteria, yeast, waste materials have been used to eliminated the use or generation of hazardous substances in the design, manufacture and applications of chemical products. The use of catalysts (polysaccharides, enzymes etc.) in place of physical and chemical reagents improve atom efficiency, reduces the cost of synthesis, free of chemical contaminants, and are safe and eco-friendly. These biomolecules play an active role in the formation of nanoparticles, through biogenic reduction of metal precursors, the nature of biological entities in different concentrations influence the shape and size, acting as driving force for the synthesis of nanoparticles.

Biogenic reduction is a “Bottom Up” approach similar to chemical reduction where a reducing agent is replaced by extract or molecules of natural or waste products with inherent stabilizing growth terminating and capping properties. The bio-medical applications including bio-imaging, drug delivery, biosensors, and gene delivery. Gopi et al. (2015) developed hydroxyapatite nanoparticles (25 nm) using pectin extracted form peels of prickly pear as template for the synthesis. The nano-hydroxyapatite particles were pure, low crystalline and showed potential

antimicrobial activity. The *in vitro* apatite formation showed an enhanced bioactivity, so the synthesized nanoparticles can act as a better bone regenerating material in the field of biomedicine. Kandasamy et al. (2019) described the green synthesis of CdS nanoparticles (spherical shaped,  $d_{50} = 9.56$  nm) using *Opuntia ficus-indica* fruit sap as a green template with extrinsic crystallite size control in the quantum confinement range.

### 9.3 Green Synthesis of Nanoparticles Using Cactus Extracts

Alvarez-Bayona et al. (2019) reported the success in synthesis of Au/Li nanoparticles using extracts from *Opuntia ficus-indica* through green synthesis. Kombaiah et al. (2017) reported the synthesis of spherical  $ZnFe_2O_4$  nanoparticles through green synthesis using *Opuntia* extract. The synthesized Zinc ferrites nanoparticles were used in the catalytic reaction for the oxidation of glycerol into formic acid with a good catalytic performance and better selectivity of formic acid throughout the reaction. Francisco-Escudero et al. (2017) applied aqueous extracts of *Opuntia amyclaea* to promote the biorreduction of metal ions and as a particle stabilizing agent in the biosynthesis of zinc oxide nanoparticles (bio-conductors). The developed zinc oxide nanoparticles showed submicron sizes with hexagonal structure. It did not show antibacterial activity (40  $\mu$ L from a 1628  $\mu$ g/mL solution) against *Escherichia coli* and *Staphylococcus aureus* due probably to the encapsulation of polysaccharides and mucilage proteins employed. The pH was critical in order to obtain NPs-ZnO free of  $Zn(OH)_2$  with low particle size.

Silva-de Hoyos et al. (2012) prepared stable silver nanoparticles (23 nm) in a colloidal aqueous solution by the chemical reaction of silver nitrate ( $AgNO_3$ ) and *Opuntia ficus-indica* aqueous extract, which function as both, reducing and stabilizing agent. Concentration of reducing agent and temperature during the process can control the size and morphology of nanoparticles. Ledezma et al. (2014) synthesized silver spherical nanoparticles (4–28 nm, an average size 10 nm) using nopal extracts as reducing agent and poly (PVA) as stabilizing agent. Silver nanoparticles were incorporated into PVA nanofiber (250 nm) by electrospinning process. Ag nanoparticles/nanofibers at 50 and 20 ppm concentrations showed antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Aspergillum niger*.

Baylon et al. (2015) correlated the chemical composition, reducing sugar and phenol compounds content, with the reduce capacity of metallic ions in order obtain silver nanoparticles. They assessed seven wild and two domestic strains of nopal *Opuntia* spp. and found significant differences in the content of this compounds in all nopal strains analyzed. The freeze-dried extracts were used to reduce an  $AgNO_3$  solution to obtain Ag-nanoparticles. The authors reported that Milpa alta, Copena, and Xoconostle extracts strains showed very high reduction rates (high nanoparticles yield), while tuna cardona and blanca, as well as rastrero nopal extracts showed from moderate to very slow reduction rates.

## 10 Bioenergy from Cactus

Faced with the challenge of responding to the world wide needs in the search for new energy sources, the nopal (*Opuntia* spp.), as a result of their chemical composition and their metabolism characteristics, presents advantages in relation to other species given that its high productive efficiency, wide adaptation range, fast growth and low input requirements which allow their grown in marginal and semiarid and arid lands. Cacti have been proposed as an energetic resource. Likewise, the bioenergy from cactus biomass is considerable inexhaustible, clean, sustainable, and constitutes an energy option viable, since from its stems and fruits it is possible to obtain biogas, biodiesel and bioethanol or semi-finished products that can be used directly (Table 37.11).

### 10.1 Bioethanol

Sanchez et al. (2009) carried out different hydrolysis pretreatments [S1-hydrolyzing fresh cladode with boiling hydrochloric acid 1 M (30 min) or S2-with concentrated sulphuric acid (96%) and autoclave at 121 °C for 20 min] and conditions of process (4 or 5 days of retention time at 25 or 30 °C) in order to determine the best procedure to obtain the maximum ethanol concentration using the yeast *Saccharomyces cerevisiae* throughout fermenting non-cellulosic carbohydrates from prickly pear

**Table 37.11** Nopal as source of biofuels

Bioethanol	Production <sup>a</sup>	Fermentation yield (%) <sup>b</sup>	References
	6041.5 mg	41	Sanchez et al. (2009)
	19.5 g L <sup>-1</sup>	66	Kuloyo et al. (2014)
	20.6 g L <sup>-1</sup>	70	Kuloyo et al. (2014)
	12.9 g L <sup>-1</sup>	49	López-Domínguez et al. (2019)
	1490–1875 L ha <sup>-1</sup> year <sup>-1</sup>	NR	Santos et al. (2016)
Biogas (Methane)	Production	Variety; Density (plants/ha)	
	1860 m <sup>3</sup>	<i>O. ficus-indica</i> ; 20,000	Krümpel et al. (2020)
	1791 m <sup>3</sup>	<i>Euphorbia tirucalli</i> ; 266,667	Krümpel et al. (2020)
	281–382 mL g <sup>-1</sup> VS <sup>-1</sup>	<i>Opuntia</i> ; NR	Lueangwattanapong et al. (2020)
	3717 m <sup>3</sup> ha <sup>-1</sup> year <sup>-1</sup>	Prickly pear; NR	Santos et al. (2016)

NR not reported

<sup>a</sup>Concentrations were below the desired level (5%) that considered as economically feasible

<sup>b</sup>Respect to initial sugar content

cladodes. Although considerable concentrations of ethanol were reached (6041.5 mg ethanol which means 41% of fermentation yield respect to initial sugar content) by S2 treatment, these concentrations were far below the desired level (5%) that considered as economically feasible.

In another study, Kuloyo et al. (2014) assessed the effects of limited aeration on the fermentation profiles, sugar utilization, and ethanol production using enzymatic hydrolysate of pretreated cladodes of *Opuntia ficus-indica* as feedstock. Ethanol concentrations up to 19.5 g L<sup>-1</sup> (66%) and 20.6 g L<sup>-1</sup> (70% of the theoretical yield on total sugar in the hydrolysate) were obtained in oxygen limited cultures with *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*, respectively. These ethanol concentration represent almost double compared to non-aerated cultures in which similar ethanol yields were reported through fermentation procedures using *K. marxianus* and *S. cerevisiae* at 30 and 40 °C. Not only ethanol yield by *K. marxianus* was enhanced, but also the utilization of galactose, xylose and arabinose. Besides, the authors reported an improvement in ethanol concentration obtained by fermentation of cladodes of 1.4% (w/v) respect to that reported by Retamal et al. (1987) who reported 1.2% (w/v) of ethanol production using fruits and cladodes. However, further bioprocess development (deal with viscosity and increase the fermentable carbohydrate concentration in the hydrolysate) is required to obtain an economically viable ethanol concentration of at least 4% (w/v) (Wingren et al., 2003) from this lignocellulosic feedstock.

López-Domínguez et al. (2019) evaluated ethanol production using cladodes flour (20%) as unique carbon source and two wild microorganism *Acinetobacter pittii* isolated from decaying cladodes at conditions of 37 °C and pH 6.5 for both total cellulases (0.67 IU/mL) and endoglucanase (0.23 UI/mL), respectively, and *Kluyveromyces marxianus* isolated from termite stomach for 4 h at 40 °C and pH 5.5 as conditions to obtain the maximum alcohol production (12.9 g/L). Based on their results, the authors suggest the use of *A. pittii* to develop the hydrolysis of carbohydrates and then, the use of *K. marxianus* for alcoholic fermentation in a simultaneous or semi-simultaneous process.

## 10.2 Biogas Production (Methane)

Ramírez-Arpide et al. (2018) assessed that nopal (*Opuntia ficus-indica* (L.) Mill.) could be used for biogas production by co-digestion with dairy cow manure. The authors took in consideration the fact that the last one is the second largest source of greenhouse gas emissions in dairy farms. Additionally, a life cycle assessment was carried out to evaluate the environmental impact and energy balance of biogas production associated with the process to evaluate the feasibility of using nopal as biogas source. The authors compared an organic farming system and a conventional farming system. Biogas production and yield data obtained in a 10 L anaerobic digester showed that the energy return on invest for biogas production ranges from 8.1 to 12.4. Organic farming system decreased the environmental impact by 22.5%



in the global warming potential category but increases the acidification potential and eutrophication potential impact category values by 47.2% and 45%, respectively, while covering the digestate tank results in 2.3% reduction in global warming potential and 1.7% reduction in photochemical ozone creation potential.

It is important to highlight that gas production from nopal cladode and dairy cow manure co-digestion and digestate management offers cleaner energy production since the global warming potential has a lower value than that reported for similar feedstocks. The use of these two biomasses combines the strengths of a plant that accumulates biomass efficiently and the reduction of greenhouse gas emissions by using one of the main wastes in dairy production.

Krumpel et al. (2020) studied the potential of intensive cultivation of *Opuntia ficus-indica* and *Euphorbia tirucalli* under various planting densities on marginal land blocks (900 m<sup>2</sup>) and compared the specific methane production of their biomass. The results showed that high density planting did not negatively affect the plant growth and biomass production. During 4 months growth time, the plantation at the highest density of *Opuntia ficus-indica* 20,000 plants/ha of marginal land was able to generate a methane yield of approximately and 1860 m<sup>3</sup>. This methane production was higher than 1791 m<sup>3</sup> generated in the case of *Euphorbia tirucalli* at 266,667 plants/ha.

Lueangwattanapong et al. (2020) reported that methane yield (281–382 mL/g VS) of five selected crassulacean acid metabolism (CAM) species from the five different genera (*Agave*, *Ananas*, *Euphorbia*, *Kalanchoe*, and *Opuntia*) were equivalent to that of the maize. Therefore, CAM plants could represent viable new feedstocks for biogas production. *Agave angustifolia* Haw. generated the highest methane yield of all CAM species. It is important to highlight that batch assays were performed using sludge and rumen fluid as inocula under uncontrolled pH at 39 °C. So, rumen fluids could play an important role in the hydrolysis step.

Lower lignin in CAM plants as compared to maize resulted in a faster volatile fatty acid (VFA) production suggesting that CAM plants may be viable as bioenergy crop. Santos et al. (2016) reported that compared with other crops, prickly pear showed high potential for methane production (3717 m<sup>3</sup> ha<sup>-1</sup> year<sup>-1</sup>) being comparable to traditional energy crops. In contrast, the ethanol yield potential (1490–1875 L ha<sup>-1</sup> year<sup>-1</sup>) was lower compared to traditional biomass sources (sugarcane and sugar beet for example).

## 11 Nopal as Source of Electricity

Deshpande and Joshi (1994) fabricated an electrochemical cell using cactus stem as an electrolyte. The authors studied the discharge characteristics and found that at a current drain of 100  $\mu$ A, the cell gives an optimum energy density of 175 mW h kg<sup>-1</sup>. The power generated by these cells is enough to run piezoelectric buzzer and a LCD calculator for a few hours.

Kamaraj et al. (2019) focused their work on spending biomass effluent made with prickly pear cacti mixed with cattle dung waste (80:10) to improve resource utilization, reduction of the waste stream and further energy production through microbial fuel cells (MFCs) system. The design of a modified clay cup (*cantarito*) microbial fuel cell (C-MFCs) improved applying commercial acrylic varnish (AV) on the internal side (In-C-MFCs), external side (Out-C-MFCs) and both sides (Both-MFCs) of the clay cup to digest the biomass effluent from nopal biogas. The maximum volumetric power density were 1841.9 mW/m<sup>3</sup>, 1023.7 mW/m<sup>3</sup>, and 448.9 mW/m<sup>3</sup> for Both, Out, and In-C-MFCs, respectively. The produced methane by decomposition of biomass effluent was used for fuel and burned to generate enough electricity in this case to power a 4-digits clock with two Both-C-MFCs connected in series for 29 days. The acryloyl group in varnish could favor the performance of C-MFCs, this theory is supported by the fact that control experiment (C-MFC without applied varnish) did not show a stable potential as a consequence a poor performance. Additionally, metagenomics studies reveal the diversity of the microbial community at the initial and final stage of MFC operation. The microbial diversity including members of the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Synergistetes* and candidate division TM7 was found. Interestingly, the microbial analysis revealed that Rhizobiales and *Bdellovibrio* were significantly more abundant in Both-C-MFCs.

Apollon et al. (2020) explored the application of plant microbial fuel cell (P-MFC) in arid or semi-arid areas using four *Opuntia* species for the generation of sustainable electricity via plant-based biobattery design under open environment and unsaturated water conditions. The authors constructed a Novel vertically integrated plug-in ceramic stick based P-MFCs, this could reduce the usage of the top-soil surface and aid the possible potential of scale-up design. For a long operating time (30 days), the results showed an average power density of 103.6 mW m<sup>-3</sup> with reactors using *Opuntia albicarpa*, followed by *Opuntia ficus-indica* (10.63 mW m<sup>-3</sup>) > *Opuntia robusta* (7.46 mW m<sup>-3</sup>) > *Opuntia joconostle* (0.46 mW m<sup>-3</sup>), with 1000 Ω resistance. Higher total electricity production of 285.12 J was achieved in *Opuntia albicarpa* over 4 weeks. In addition, the energy generation of 3.66 W h m<sup>-2</sup> was achieved in this study. Notably, *Opuntia ficus-indica* and *Opuntia albicarpa* species showed a significant height in the first 2 months (P-value < 0.05). The authors concluded that: this finding opened the avenue for the electricity generation impact on plant growth. The P-MFC also shows the potential to be used in a semi-arid area.

## 12 Nopal Used in Solar Cells and Holographic Materials

Olivares-Pérez et al. (2012) described the use of 0.8 mL fermented nopal (*Opuntia ficus-indica*) extracts mixed with 2.5 mL polyvinyl alcohol (PVA-matrix), deposited by a gravity technique on a glass substrate, as a recording medium to be applied in

holographic grating, written by a He-Cd laser (442 nm). The nopal extract used was generated through the degradation of pectic substance and chlorophyll by natural processes of fermentation through sugars and alcohols. Despite temperature has an important role in the decomposition rates of chlorophyll and mucilage through pheophytins reactions, the fermentation was developed at room temperature, a process in which is highly likely that the Mg atoms in most chlorophyll nuclei are substituted by  $\text{Fe}^{2+}$  generating a dark brown color from the chlorophyll fermented mucilage. The authors demonstrated the presence of Fe-pheophytin, together with a mixture of combination of many other mineral ions responsible for the photosensitivity of the material. Previous studies have written holograms with Fe (III), which makes a transition to Fe (II) for recording an image. Even though the PVA film with the fermented extract is dried to form a photosensitive emulsion, the extract is strongly affected by the environmental conditions, which determine the speed of the aging process of the photosensitive film (oxidation). The photosensitive material developed behaved well, under normal laboratory conditions (room temperature 20 °C, relative humidity 40%) has the property of self-developing, is easy to handle, has a low cost, low toxicity and showed adequate properties for building transmission holographic gratings. Besides, the holographic elements constructed from this material have a diffraction efficiency of approximately 32.3% to first order at the Bragg angle.

Ganta et al. (2017) developed a dye-sensitized solar cell (DSSC) using as sensitizers natural dyes (chlorophyll and anthocyanin adhesion promoters) extracted from the cladode of prickly pear (*Opuntia ficus-indica*), the gel of Aloe Vera (*Aloe barbadensis miller*), and the combination of cladode and Aloe Vera extracts on side-by-side configuration. These adhesion promoters helped in efficient adsorption of plant dyes onto the  $\text{TiO}_2$  film, leading to photoelectric conversion. The photoelectrochemical performance of DSSCs revealed a open circuit voltages ( $V_{oc}$ ) ranged from 0.440 to 0.676 V. Fill factors (FF) were greater than 40%, short-circuit photocurrent densities ( $J_{sc}$ ) oscillated from 0.112 to 0.29  $\text{mA}/\text{cm}^2$  and the highest conversion efficiency of 0.74% was reported for the Cladode DSSC due to the presence of stronger adhesion promoters in the chlorophyll dye, providing a better charge transfer. Moreover, the authors overcome the problem in mixing the dyes by designing a DSSC using two dyes on a side-by-side configuration (conversion efficiency of 0.50%). Due to their low production cost, simple and energy-efficient assembly method, and environmental friendliness, this natural plant bases dyes as sensitizers of DSSCs are promising (Ganta et al., 2017).

Purushothamreddy et al. (2020) explored prickly fruit extracts as a photosensitizer in DSSC and confirmed the presence of betacyanin in the extract and hydroxyl groups anchoring onto the  $\text{TiO}_2$  surface. Reflectance edge of  $\text{TiO}_2$  is red-shifted upon the adsorption of natural dye. The fabricated DSSC had a conversion efficiency ( $\eta$ ) of 0.56% with high fill factor (FF) of 0.85, open-circuit voltage ( $V_{oc}$ ) of 0.56 V, and short circuit-current density ( $J_{sc}$ ) with 1.17  $\text{mA}/\text{cm}^2$ .

### 13 Bio-Functional Foods

The demand of functional and probiotic foods is on the rise and there is always a quest to develop array of the products rather than the traditional products. Prickly pear stem (nopal) has been used in folk medicine and a raw material since ancient times. Stems have been proved to possess components with valuable biological activities. Nowadays, people consume food not only to cover the nutritional requirements, they also demand for healthy, natural and convenient food that show biological activities. The addition of mucilage from nopal to food products could generate functional food (Table 37.12).

**Table 37.12** Nopal based biofunctional foods

Product	Biofunctionality	Compounds related	References
Tortillas	Prebiotic activity	Soluble fiber (mucilage and pectin)	Guevara-Arauza et al. (2011)
	Improve oxidative status	Flavonoids (Quercetin; dihydroquercetin)	
	Antioxidant activity	Chlorophyll, betacyanins; betalain	
	Improve digestive system	Insoluble fiber (cellulose and lignins)	
	Improve immune system	Soluble fiber consumed by acidolactic bacteria	
Bread rolls	Prebiotic activity	Soluble fiber (mucilage and pectin)	Guevara-Arauza et al. (2015), Guevara-Arauza et al. (2012)
	Antioxidant activity	Chlorophyll, betacyanins; betalains	Guevara-Arauza et al. (2011), Bouazizi et al. (2020)
Juice and beverage	Antioxidant activity	Flavonoids, chlorophyll, betacyanins; betalain	Panda et al. (2017)
Ice cream	Prebiotic activity	Soluble fiber (mucilage and pectin)	Guevara-Arauza et al. (2012)
	Antioxidant activity	Chlorophyll, betacyanins; betalain	Perez-Navarro (2016)
	Improve digestive system	Insoluble fiber (cellulose and lignins)	Guevara-Arauza et al. (2011)
	Improve immune system	Soluble fiber consumed by acidolactic bacteria	El-Samahy et al. (2009)
Salami	Antioxidant activity	Betalain pigments	Kharrat et al. (2018)
	Prebiotic activity	Soluble fiber (mucilage and pectin)	

### 13.1 *Tortillas*

Guevara-Arauza et al. (2011) studied the bio-functional effects of nopal supplemented products, tortillas and bars (filled with prickly pear fruit jam) and reported that these kind of products have suitable physicochemical characteristics to be marketed. Interestingly, a high content of bioactive components (i.e., dietary fiber and polyphenols) were achieved in the products. Daily supplementation with nopal-based tortilla over 21 days, improved the oxidative status of healthy humans. Experimental evidence showed increased levels of trolox equivalent antioxidant capacity (TEAC, 1.47 mmol/L), phenolics (7.67 mg QE/L) and vitamin C (77.91  $\mu$ mol/L) in volunteer's plasma after intake and the decreased oxidative status in plasma lipids (MDA levels), as well as lower concentrations of the main antioxidant agent in the oxidized form in erythrocytes (GSSG). In addition, a diminution of glucose (4.43 mmol/L), cholesterol (total 4.27 mmol/L, and LDL, 1.96 mmol/L), and triglycerides levels (1.54 mmol/L) were detected, which together reduce the risk of various chronic diseases. The relative low levels of antioxidants provided by the nopal-based products can not generate the observed effects by themselves. Therefore, other compounds with antioxidant activity present in nopal and compounds of the formulation should be involved in the observed findings. The author concluded that the intake of nopal-based tortillas with high content in fiber and antioxidants could help to improve the overall oxidative status in humans, which can reduce the risk of some chronic diseases. In addition, these products showed suitable physicochemical characteristics to be marketed (Guevara-Arauza et al., 2011).

### 13.2 *Bread Rolls*

Guevara-Arauza et al. (2015) assessed the addition of total fiber (TF), insoluble fiber (IF), and soluble fiber (SF) from nopal to wheat flour to make bread rolls. The rheological properties of dough as well as quality, texture, sensorial and physical characteristics of the crumb rolls produced were evaluated. The storage (23.5 MPa) and loss modulus (11.9 MPa) for SF-dough were the lowest indicating that a less visco-elastic behavior was obtained. Polarized light microscopy showed that a more homogeneous size and a better distribution of starch granules were developed into SF-dough. Crumb hardness (3.25–4.78 N) and chewiness (0.31–0.81 N) of SF-rolls were lower than the control experiment (3.99–5.81 N and 0.35–1.01 N, respectively). Springiness for all treatments was constant (1.0) compared with the control (1.02–0.87) for 2 days of storage. The lowest cohesiveness values (0.24–0.14) were computed by IF treatment for a similar storage time. The specific crumb volume increased by 12.46%, 9.03%, and 1.10% by the addition of SF, TF and IF, respectively. The lowest rate of staling was shown by SF-rolls (0.199), and it was followed by TF (0.296), IF (0.381) and control (0.458) treatments. As a result, the highest

scores on quality (9.3 out of 10) and sensorial attributes (from 8.9 up to 9.7) were assigned to SF-rolls. The authors concluded that the addition of soluble fiber produced the softest dough. Therefore, a less visco-elastic behavior was obtained and a better distribution of starch granules was developed into SF dough. These results agreed with crumb texture profile analysis, where the lowest hardness and chewiness were shown by SF-roll crumb. In addition, rolls formulated with SF showed the lowest rate of staling, had excellent bread acceptability and showed an increase in specific volume. On the other hand, the addition of total fiber and insoluble fiber developed harder dough than the control experiment, accordingly higher crumb hardness and chewiness were recorded. These improvements on rolls may be considered additional to the nutritional benefits of nopal soluble fiber which has been shown. It has prebiotic activity (Guevara-Arauza et al., 2012), so further studies should be addressed in order to determine the possible bio-functionality of these rolls.

Bouazizi et al. (2020) did successfully integrate prickly pear peels flour “PPPF” (20 g/100 g) as key ingredient in biscuits formulation. The main bioactive compounds in prickly pear biscuits are betalains (2776 mg/100 g d.w.), fibre (20.70 g/100 g d.w.), and considerable ash levels (14.57 g/100 g d.w.). The authors highlight the improving on technological properties such as the aptitude to kneading, the flavor retention, and the antioxidant capacity after addition of PPPF. Likewise, the acceptance sensory test showed that biscuits prepared with 20 g/100 g and 30 g/100 g PPPF computed the better score for smell, colour and overall acceptability.

### 13.3 Juice and Beverage

Panda et al. (2017) prepared a prickly pears-lacto juice by fermenting the juice of prickly pears as substrate (diluted by appropriate factor) with strain *Lactobacillus fermentum* ATCC 9338. The overall acceptability of developed product was recommended by sensory panelist. The composition and properties of lacto-juice were: ascorbic acid, 6 mg/100 mL, DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, 105 $\mu$ MTE (micro molar trolox equivalent)/mL; total soluble solids, 5.9°Brix; total sugar, 1.75 g/100 mL; reducing sugar, 0.20 g/100 mL; pH, 4.1; titratable acidity, 1.7 g tartaric acid/100 mL; lactic acid, 0.32 mg/100 mL; and phenol, 0.41 $\mu$ g/mL. The authors highlighted the disintegration during the course of probiotic fermentation of some risky organic compounds such as 4h-Pyran-4-one, 3,5-dihydroxy-2-methyl; furfuryl alcohol; 2-propenenitrile, 2-(acetyloxy); 2,2-diethyl-3-methyloxazolidine; acetaldehyde and furan present in the fresh fruit juice.

### 13.4 Ice Cream

Guevara-Arauz et al. (2012) reported that mucilage (MO) and pectic-derived (PO) oligosaccharides from prickly pear stems could act as prebiotic. The research indicates that a mixture of MO treatment enhanced lactobacilli growth up to 23.8%, while PO increased the bifidobacteria population by 25%. Furthermore, the addition of MO produced a slight decrease in *enterococci*, *enterobacteria*, *staphylococci*, and *clostridia* of about 4%. Increased levels of the short-chain fatty acids (SCFA) were attained in the cultures at rates of 35% and 16% in response to MO and PO treatments, respectively. Propionic acid (propionate) and butanoic acid (butyrate) production increased at least 50% throughout MO and PO treatments. A decrease in the ammonium level of 11.5% was produced by MO treatment.

Some attempts were done to incorporate prickly pear pulp to ice cream and water ice. Perez-Navarro (2016) incorporated orange and purple prickly pear pulp to ice cream (25% and 20%) and water ice (40% and 35%). In these products, the total betalain content was 635 mg L<sup>-1</sup> and 990 mg L<sup>-1</sup> for prickly pear pulp, 46.93 mg L<sup>-1</sup> and 96.23 mg L<sup>-1</sup> for water ice, and 20.4 mg L<sup>-1</sup> and 39.5 mg L<sup>-1</sup> for ice cream added with orange and purple prickly pear pulp, respectively. The Trolox equivalent was 860 and 1213 for prickly pear pulps, 65.3 and 56.6 for water ice, and 60.2 and 39.5 (μmol Eq Trolox 100 mL<sup>-1</sup>) for ice cream formulated with orange and purple, respectively. In addition, the overrun was 18.1% and 7.53% for water ice, and 30.2% and 16.9% for orange and purple ice cream, respectively. About sensory product acceptance, the orange ice cream was the most preferred.

El-Samahy et al. (2009) tested the addition of red cactus pear (*Opuntia ficus-indica*) concentrate up to 30°Brix and four levels (0%, 5%, 10%, and 15%) to basic ice cream mix (0.5% gelatin, 8% fat, 10.5% milk solids non-fat (MSNF), and 16% sucrose). The rheological properties of all ice cream mixes before and after aging showed that the flow behavior of mixes is non-Newtonian besides being pseudo-plastic behavior. While specific gravity and weight per gallon of resultant ice cream samples increased by increasing of added pulp. Sensory evaluation of ice cream samples showed that sample with 5% cactus was very desirable and very close to control sample.

### 13.5 Salami

In order to replace some synthetic additives by a natural extract from red prickly pear (*Opuntia stricta*) in salami, Kharrat et al. (2018) added 2.5% of prickly pear extract (PPE), as natural colorant and antimicrobial agent in salami formulation. They found a decrease in hardness and chewiness of the formulated salami. The sensory panel appreciated more the color, taste and texture of salami prepared with 2.5% PPE. Moreover, PPE inhibited bacterial growth in salami stored at 4 °C, over 30 days. Prickly pear extract (PPE) displayed a strong antioxidant and antimicrobial

activities associated likely to its high level of phenolics (152.2 $\mu\text{g}$  QE/mg PPE), flavonoids (370.6 $\mu\text{g}$  GAE/mg PPE) and carbohydrates content (18.8%) that composed mainly of galactose, rhamnose and galacturonic acid. Overall, the betalain pigment, carbohydrate and phenolic compounds present in PPE could be used as a natural colorant, antioxidant and antimicrobial agent without change of the sensory characteristics in meat products.

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# Chapter 38

## Bread Enrichment with *Opuntia* spp. Derivatives



Hülya Gül

**Abstract** Bread, basic food in many parts of the world, is usually prepared by mixing wheat flour, water, salt, and yeast in varying proportions. Apart from these primary components, incorporating functional ingredients that provide health benefits into bakery products has overgrown due to the increasing demands of consumers for functional food. Several studies have shown that bread, which contains functional ingredients, particularly dietary fiber, and antioxidants are associated with maintaining health and decreased risk of some chronic and degenerative diseases, obesity, inflammatory and aging. Many functional ingredients are derived from natural raw materials, cereal-milling by-products, fruit and vegetables, and their processing by-products, pseudocereals (buckwheat, quinoa, and amaranth), legume flours, and oilseeds can be introduced in bread. *Opuntia* spp. derivatives are an attractive alternative to meet the actual trend for new sources used as a functional ingredient. Some research demonstrates that many parts of *Opuntia* spp. such as cactus pear fruits, stems (cladodes), and fresh mucilage have a vast potential to be used in bread making. *Opuntia* species include high values of dietary fiber, protein, minerals, vitamins, and antioxidants. These nutrients have a protective ability against cancer, cardiovascular disorders, hypercholesterolemia, inflammatory, obesity, and allergic diseases. Using *Opuntia* spp. derivatives can provide healthy bread with higher nutritional quality such as more protein content, ash, dietary fiber, phenolics, antioxidants, and oxidative stability than the wheat bread. However, the inclusion of fiber-rich ingredients, up to a certain level, impair dough rheology and bread quality, including sticky dough, reduced loaf volume, increased crumb hardness, and coarse texture dark-colored crumbs, and taste alterations. This chapter evaluates the impact of *Opuntia* spp. derivatives on the dough rheological properties as well as the physical, nutritional, technological, textural, and sensory aspects of bread.

**Keywords** Rheology · Bread quality · Bioactive compounds · Functional properties · Sensory

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## Abbreviations

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
DW	Dry weight
FAO	<a href="#">Food and Agriculture Organization</a>
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
LDL	Low-density lipoprotein
TE	<a href="#">Trolox equivalent</a>

## 1 Introduction

Cereals and cereal-flours-based food are the main components of the human diet worldwide since ancient times. World cereal grain production reached nearly 2963 million tonnes in 2018, according to the latest data reported by the [Food and Agriculture Organization](#) (FAOSTAT, 2020). Among cereals, maize, wheat, rice, sorghum, millet, barley, oat, and rye are most widely cultivated to produce essential foodstuffs such as bread, pasta, noodle, biscuits, cake, gluten-free products, and breakfast cereals. Cereals are the primary energy source due to the high content of carbohydrates, complex sugars, protein, dietary fiber, minerals, and B-vitamins (Serna-Saldivar & Barbosa-Canovas, 2010).

Cereal-based products, especially bread, are suitable nutritional components that lead to functional foods due to their vast consumption worldwide. Many studies focused on the enrichment of bread with various ingredients rich in dietary fibers, antioxidants, and phenolic substances. For example, legume flours, fruit or vegetable products, cereals and seeds, cereal-milling by-products, spices, herbs, and parts of green plants were used (Dziki et al., 2014; Hemdane et al., 2015; Fendri et al., 2016; Martins et al., 2017; Pathak et al., 2017; De Lamo & Gómez, 2018; Gumul et al., 2019; Torbica et al., 2019; Betoret & Rosell, 2020).

A remarkable increase in dietary fiber, phenolics, and flavonoids for bread with an added 5% lavender and melissa waste can be obtained (Vasileva et al., 2018). Świeca et al. (2017) detected higher phenolics and antioxidant activity when bread was supplemented with 5% green coffee. A higher level of phenolic compounds and dietary fiber also have been reported in bread enriched with chia (*Salvia hispanica*) seeds (Romankiewicz et al., 2017), pomegranate seed flour (Gül & Şen, 2017a), rosehip seed flour (Gül & Şen, 2017b), hemp flour (Mikulec et al., 2019), yam (*Dioscorea opposita* Thunb.) flour (Li et al., 2020), aerial parts of sweet potato (Mau et al., 2020), and black carrot (*Daucus carota* ssp. *Sativus* var. *Atrorubens alef*) fiber (Yılmaz & Pekmez, 2020).

The incorporation of functional ingredients derived from *Opuntia* spp. which have high bioactive nutrients and functional properties (Arias-Rico et al., 2020), into bakery products became a popular trend. The beneficial potential of cactus for

nutrition and health has been put forward by researchers and private sectors (El-Mostafa et al., 2014). *Opuntia* spp. is a cactus species of the *Opuntia* genus, belonging to **Cactaceae's family** with about 1500 species (El-Mostafa et al., 2014). *Opuntia* spp. is commonly found in the non-productive agricultural lands, particularly in arid and drought-affected regions of the World (Barba et al., 2017), and have a remarkable adaptation to high temperature, poor-quality soils, UV radiation, and drought (Oniszczuk et al., 2020).

Fruits (prickly pears), stems (cladodes), flowers, and leaves of *Opuntia* spp. are edible and used as food, water sources, and medicine in traditional treatments. In recent years, usage of *Opuntia* spp. in the nutraceutical, cosmetic, personal care, food, feed, and bioenergy industries have become popular due to its **nutritional, functional, therapeutic** impacts (Barba et al., 2017; Ciriminna et al., 2019; Gouws et al., 2019). In this respect, *Opuntia* spp. compounds and derivatives can be shown as a natural ingredient for cancer chemoprevention (Zou et al., 2005), antimicrobial activity (Ammar et al., 2012; El-Mostafa et al., 2014; Khemis et al., 2016; Melgar et al., 2017), anti-inflammatory effects (Antunes-Ricardo et al., 2015), the therapeutic effect for metabolic syndrome, rheumatism, non-alcoholic fatty liver disease, alcoholism, cerebral ischemia, anti-hypercholesterolemic properties, antiviral and antispermatogenic effects (El-Mostafa et al., 2014; Ventura-Aguilar et al., 2017). Functional bread developed from *Opuntia ficus-indica* fruits powder showed reducing effect on the body's glucose level and weight loss and exerted an antioxidative effect on induced oxidative stress in streptozotocin-treated diabetic rats (Moon et al., 2012). All of these health benefits have arisen from the rich composition of *Opuntia* in antioxidant compounds (phenolics, flavonoids, betaxanthins, and betacyanins), vitamins (ascorbic acid, vitamin E), carotenoids, fibers, polyunsaturated fatty acids, tocopherols, sugars, organic acids and amino acids (El-Mostafa et al., 2014; Morales et al., 2014; Melgar et al., 2017).

*Opuntia* spp. are characterized by their higher antioxidant activity due to the variety of phenolics, flavonoids, and betalains (Butera et al., 2002; Morales et al., 2014) content. A high potential of DPPH free radical scavenging (99.7%), and total phenolics (14.9 mg GAE/g dry matter) content of *Opuntia ficus indica* fruit was reported by Oniszczuk et al. (2020). *Opuntia* fruit (prickly pear) are rich source of free phenolic acids, mostly benzoic acid derivatives: syringic, protocatechuic, 4-OH-benzoic, salicylic, vanillic, gentisic, and cinnamic acid derivatives: *trans*-sinapic, caffeic and *cis*-sinapic, ferulic, *p*-coumaric, 3,4-dimethoxycinnamic, *m*-coumaric and isoferulic acids.

The genus *Opuntia* may be used in the preparation of several food products, which include: marmalades, juices, jam, tea, beverages, milk-based drinks, flours, breakfast cereals, bread, pasta, cookies, cakes, gluten-free products, pigment, and pectin (Sawaya et al., 1983; Stintzing & Carle, 2005; Moßhammer et al., 2006; Leopoldo et al., 2012; Lefsih et al., 2017). While bread may be an excellent food carrier of added *Opuntia* spp. derivatives. In this frame, this chapter presents:

- Enrichment of bread and bakery products with *Opuntia* spp. derivatives
- Composition of functional ingredients obtained from *Opuntia* spp.

- The effect of *Opuntia* spp. derivatives on the rheological properties of bread dough
- The effect of *Opuntia* spp. derivatives on the physical, textural, technological nutritional, and sensory aspects of bread and flour-based bakery products

## 2 Bread Enrichment with *Opuntia* spp. Derivatives

Bread is one of the most important part of the human diet worldwide. Principal constituents of bread are wheat flour and/or cereal-based flours, salt, water, and yeast. Although bread provides energy, protein, minerals, and other macro and micronutrients, some bioactive components and essential amino acids are not present. Meanwhile, incorporating beneficial ingredients in bread becomes increasingly popular. Because of its low cost, wide availability, and daily consumption, bread is a suitable food for developing new and innovative functional food. Functional ingredients (powder and/or extract of *Opuntia* spp. plant or its fruits, cladodes, or seeds) have been derived from *Opuntia* spp. Several studies (Table 38.1) evaluated the potential use of the *Opuntia* spp. derivatives to the formulation and production of functional bread. Moreover, several international patents were published related to bakery products and pasta enriched with *Opuntia* spp. (Juarez et al., 2005; Garza-Lopez et al., 2006; Cornelli, 2016). One of these inventions (Cornelli, 2016) reported that food products prepared from flour and Nopal (*Opuntia ficus-indica*) showed weight loss, decrease lipids, plasma LDL cholesterol, and glucose. Powder from *Opuntia ficus-indica* (Shin & Lee, 2005), cactus Chounnyuncho (*Opuntia humifusa*) (Kim et al., 2007, 2012), cladodes (stems) from *Opuntia boldinghii* Britton et Rose (Moreno-Álvarez et al., 2009), prickly pear cactus stem (nopal) from *Opuntia ficus-indica* plants, cv. *Milpa Alta* (Guevara-Arauz et al., 2011), cactus pear cladode flour from *Opuntia ficus-indica*, and *O. Robusta* (De Wit et al., 2015), dietary fiber from the prickly pear cactus stems of the *Opuntia ficus-indica* cv. *Milpa Alta* (Guevara-Arauz et al., 2015), cladodes from *Opuntia ficus-indica* f. *inermis* (Msaddak et al., 2017), cactus pear seed flour from *Opuntia ficus-indica* (Reda & Atsbha, 2019), seeds flour from prickly pear (*Opuntia ficus-indica*) fruit (Ali et al., 2020) and mucilage from *Opuntia ficus-indica* (L.) Mill. (Liguori et al., 2020) can be used as a functional ingredient in bread and/or other bakery products.

As can be noted from Table 38.1, functional ingredients obtained from *Opuntia* spp. can be used in powdered (floured) form or as mucilage in bread formulations. Generally, drying using various methods (atmospheric conditions, convection oven, sun drying, freeze-drying, or roasting) until reaching a certain moisture content then grinding and sieving are applied to obtain powder (flour) form of a plant, fruits, peels, stems and/or seeds of *Opuntia* spp.

**Table 38.1** Examples from the enrichment of bread with *Opuntia* spp. derivatives

Ingredient type	Preparation of ingredient form	Quantity	Final product	Determination	Reference
Prickly pear ( <i>Opuntia ficus-indica</i> ) powder	–	0	Bread	– Dough farinographic profile	Shin and Lee (2005)
		1.0%		– Rapid visco analysis	
		2.0%		– Bread Texture	
		3.0%		– Dough volume	
		4.0%		– Specific loaf volume	
Cactus (Chounnyuncho; <i>Opuntia humifusa</i> ) powder	–	–	Bread	– Dough farinographic profile	Kim et al. (2007)
				– Rapid visco analysis	
				– Fermented volume	
				– Baking loss	
				– Sensory evaluation	
Cactus pear ( <i>Opuntia boldinghii</i> Britton et Rose) stem flour	Drying (44 °C to 10% moisture content)	0	Bread	– Dough farinographic profile	Moreno-Álvarez et al. (2009)
	– Grinding, sifting (particle size of 210µ)	5%		– Proximate and microbiological bread characterisation	
		10%		– Sensory evaluation	
		15%			
		20%			
Ground prickly pear cactus ( <i>Opuntia ficus-indica</i> plants, cv. <i>Milpa Alta</i> ) stem (nopal)	– Blanching (90 °C, 15 s)	48%	Tortilla	– Physicochemical analysis	Guevara-Arauz et al. (2011)
	– Despining			– Color	
	– Grinding			– Hardness	
				– Oxidative status indicators	

(continued)

**Table 38.1** (continued)

Ingredient type	Preparation of ingredient form	Quantity	Final product	Determination	Reference
<i>Opuntia robusta</i> (cultivar “Monterrey”) flour.	– Sun drying (7–10 days)	0	Seed Bread	– Physical analysis	De Wit et al. (2015)
	– Milling in to fine flour	2%		– Chemical analysis	
		4%		– Sensory analysis	
		6%			
		8%			
		10%			
17%					
Dietary fiber fractions obtained from prickly pear cactus ( <i>Opuntia ficus-indica</i> plants, cv. <i>Milpa Alta</i> ) stems	– Obtaining dietary fiber (total, insoluble and soluble, TF, IF, SF) of prickly pear cactus according to the AOAC official method 991.43.	TF: 3.6%	Bolillo (bread roll, in Mexico)	– Dough rheological assays (with a rheometer)	Guevara-Arauz et al. (2015)
	– Freeze drying	IF: 2.18%		– The morphology of the starch granules (with a polarised light microscope)	
		SF: 0.36%		– Crumb texture analysis	
			– Roll specific volume and density		
			– Rate of staling		
			– Sensory analysis		

(continued)



**Table 38.1** (continued)

Ingredient type	Preparation of ingredient form	Quantity	Final product	Determination	Reference
Cladodes ( <i>Opuntia ficus-indica</i> f. <i>inermis</i> ; spineless cladodes) powder	Washing, cutting, drying (at 50 °C during 6 h in a convection oven), grinding, sieving through 250µm sieve	0	Flat bread	– The dough alveographic properties— Physical properties (bread volume, specific volume, bread yield, mass loss, colour parameters of crust and crumb)	Msaddak et al. (2017)
		2.5%		– Total phenolics content	
		5%		– Antioxidant activity	
		7.5%		– Sensory evaluation	
		10%			
Cactus pear ( <i>Opuntia ficus-indica</i> ) seed flour	Drying (in atmospheric conditions)	Six different formulation ratios between 0% and 25%	Traditional bread “Himbasha”	– Proximate composition analysis	Reda and Atsbha (2019)
	– Decortication using mortar and pistil to remove the sticky-remnant pulps and the seed coat			– Evaluation of sensory attributes	
	– Grinding to powder				
	– Passing through 0.45 mm sieve				

(continued)

**Table 38.1** (continued)

Ingredient type	Preparation of ingredient form	Quantity	Final product	Determination	Reference
Prickly pear ( <i>Opuntia ficus-indica</i> ) fruit seed flour	Washing and drying (60 °C for 48 h) of seeds	0	Bread	– Proximate composition analysis	Ali et al., 2020
	– Roasting (in an electric muffle furnace at 200 °C for 15 min),	2%		– Total phenolic content	
	– Grinding and passing through a 150 mesh sieve	4%		– Total flavonoid content	
		6%		– Antioxidant activity	
		8%		– Specific volume of the loaf	
10%		– Sensory characteristics			
<i>Opuntia ficus-indica</i> mucilage	– Removing cladodes chlorenchyma with a peeler	150 mL <i>O. ficus-indica</i> mucilage added to obtain 406 g dough.	Bread	– Weight loss	Liguori et al. (2020)
	– Slicing and cooking in a microwave oven (900 W) for 3–5 min			– The height	
	– Mixing with mixer Homogenizer to aid the mucilage extraction.			– Image analysis	
	– Centrifugation, decantation			– Crust and crumb color	
	– Natural mucilage unadulterated by chemicals is obtained.			– Bread firmness	
				– Sensory evaluation	
– Total phenolic content					
			– antioxidant activities (ABTS and FRAP)		

### 3 Proximate Composition of *Opuntia* spp. Derivatives

Powdered parts of *Opuntia* spp. such as whole fruit, seeds, stems, pear peel, or mucilage could be used as a potential ingredient in bread and other bakery products. In this context, the proximate composition of these potential ingredients differ depending on the part obtained from the plant. Gray or tan-colored seeds about 3 mm in diameter, thickened discoid, slightly pubescent are present in the prickly pear fruit. The seeds are nutritionally essential with their higher nutrient content. *Opuntia* spp. seed flour is prepared by separating the seeds from the fruit and drying naturally in atmospheric conditions or artificially in an electric oven then grinding and sieving to particular particle size (Reda & Atsbha, 2019; Ali et al., 2020). The seed flour is used in bread formulations as a functional ingredient because of its higher content of bioactive compounds, protein, dietary fiber, minerals, lipids, and carbohydrates.

Seed flour of prickly pear (*Opuntia ficus-indica*) has higher DPPH radical-scavenging activity (62.7%), total phenolics (5.74 mg of GAE/g), and total flavonoid (1.025 mg/g) than wheat flour (Ali et al., 2020). Ethiopian cactus (*Opuntia ficus-indica*) pear seed flour has high amounts of flavonoids and phenolic compounds 0.19 mg/100 g and 90.2 mg/100 g, respectively. *Cactus Opuntia dillenii* seeds powder has higher total phenolics, total flavonoids, DPPH and ABTS (39.56 mg 100 g<sup>-1</sup>, 595.0 mg 100 g<sup>-1</sup>, 7.47% and 108.2 μmol trolox 100 g<sup>-1</sup>, respectively) than the rice flour (31.66 mg 100 g<sup>-1</sup>, 309.5 mg 100 g<sup>-1</sup>, 2.56%, 48.95 μmol trolox 100 g<sup>-1</sup>, respectively) on dry weight basis (Reda & Atsbha, 2019).

Seed flour derived from *Opuntia macrorhiza* Engelm fruits (Chahdoura et al., 2018) contain a high amount of gluten (2.05%) and amylose (8.12%) than cladodes flour, but have a low amount of cellulose (8.62%), while both of them contain the same amounts of lycopene (0.04 mg/100 g dw). Flour of cladodes is remarkable with its higher cellulose (53.4%) content than both seed flour and commercial flour (8.6% and >1.5%, respectively).

Cactus pear seed flour has moisture range between 4.17 and 6.1, crude protein 4.78–4.63, crude fat 5.0–10.5, crude fiber 12.5–52.8, dietary fiber 50.8, total ash 1.27–3.56, total carbohydrates 23.2–55.4 (g/100 g DW basis) and total energy 392.8 kcal/100 g (Özcan & Al Juhaimi, 2011; Rayan et al., 2018; Reda & Atsbha, 2019; Ali et al., 2020). The low water content of cactus pear seed flour and/or nopal flour (7.15%) makes it easy to mix with other flours such as wheat flour (Sáenz, 1997).

Major constituent in *Opuntia* spp. seed flour is dietary fiber according to proximate composition reports. Nopal flour gains value as a source of dietary fiber due to its high fiber (42.9% total dietary fiber) content (Sáenz, 1997). However, the dietary fiber content of prickly pear seed flour is almost in the same range as pomegranate seed flour (Gül & Şen, 2017a), but lower than the dietary fiber content of fig seed

flour (Ulutürk, 2018), grape pomace seed flour (Gül et al., 2013) and rosehips (*Rosa* spp.) seed flour (Gül & Şen, 2017b).

*Opuntia* spp. seed flour also a good resource of various minerals such as potassium (532.7 mg/kg), calcium (471.2 mg/kg), magnesium (117.3 mg/kg), and phosphorus (206.18 mg/100 g dry basis) (Özcan & Al Juhaimi, 2011; Reda & Atspha, 2019). High levels of calcium (268.5 and 674.8 ppm), potassium (346.7 and 676.1 ppm), and phosphorus (1173.6 and 1871.3 ppm) content were also reported by Al-Juhaimi and Özcan (2013) in *Opuntia ficus-indica* seed flours.

Prickly pear stem (cladode) flour and cladodes powder are other alternative functional ingredients used in bread production due to their valuable composition and nutritional value. The moisture, protein, ash, and dietary fiber content of the stem (cladode) flour are within the values 10.0%, 7.43%, 28.9%, and 41.5%, respectively (Moreno-Álvarez et al., 2009). *Opuntia ficus-indica* cladodes powder contains flavonoids (quercetin 3-*O*-glucoside, quercetin, kaempferol 3-*O*-glucoside, kaempferol, kaempferol 3-*O*-rutinoside, isorhamnetin 3-*O*-glucoside, isorhamnetin 3-*O*-neohesperidoside, isorhamnetin, 3,3',4',5,7-pentahydroxy-flavanone) phenolics (zataroside-A and coumaric acid), terpenoid ( $\beta$ -sitosterol) and alkaloid (indicaxanthin). Some of these compounds have potent antioxidant potential (Msaddak et al., 2017).

Mucilage from *O. ficus-indica* cladodes contain potential ingredients, including total phenolics (122.2 mg GAE 100 g<sup>-1</sup> FW) and antioxidants (ABTS: 31.5  $\mu$ mol TE 100 g<sup>-1</sup> FW; FRAP 1.92 mg GAE 100 g<sup>-1</sup> FW). It can also be used safely in yeast or sourdough bread production since it has no inhibitory effect on yeast and lactic acid bacteria (Liguori et al., 2020).

Cladodes powders, with their technological potentiality, could be valorized in bread and other bakery products. Water solubility index, swelling, water holding capacity, and fat absorption capacity of Tunisian spiny (*Opuntia ficus indica* f. *amylocea*) and spineless cladodes (*O. ficus indica* f. *inermis*) ranges between 7.36 cm<sup>3</sup>/g and 7.78 cm<sup>3</sup>/g, 5.23–27.84%, 6.85–3.15 g water/g DM and 1.29–1.31 g fat/g DM, respectively (Ayadi et al., 2009).

Huge amounts of *Opuntia ficus-indica* pear peel arise during cactus pear pulp-based products as a waste which is another functional additive that can be used in bakery products. In the cactus pear peel powder 9.9% ash, 3.5% protein, 1.2% fat, 85.3% total carbohydrate, 6.66 g/kg calcium, 22.0 g/kg potassium, 3.71 g/kg magnesium, 2243 ppm total phenolic compounds, and also 25.9% glucose, and 21.3% fructose were measured (El-Shahat et al., 2019).

Arias-Rico et al. (2020) performed a study on powdered (obtained by freeze-drying) whole xocconostle fruits (with peel and seeds) derived from *Opuntia xocconostle* F.A.C. *Weber in Diguet* cv. *Cuaresmeño* and *Opuntia matudae* Scheinvar cv. *Rosa cultivars*. High amounts of dietary fiber (30.8–36.8 g/100 g dm) were found in dried samples, while low amounts of fat (2.5–11.9 g/100 g dm), proteins (4.0–4.8 g/100 g dm), and ashes (11.4–11.7 g/100 g dm) for *Weber in Diguet* cv. *Cuaresmeño* and *Opuntia matudae* Scheinvar cv. *Rosa*, respectively were reported. On the other hand, high concentrations of total phenolics (1580–1068 mg GAE/100 g dm) and antioxidant activity were determined by ABTS<sup>+</sup> (3261–1348  $\mu$ mol TE/100 g

dm) and DPPH (3318–1753  $\mu\text{mol TE}/100 \text{ g dm}$ ) for *Weber in Diguet* cv. *Cuaresmeño* and *Opuntia matudae* Scheinvar cv. *Rosa*. powders, respectively.

#### 4 Effect of *Opuntia* spp. Derivatives on Rheological Properties of Bread Dough

Dough rheology can be defined as the science of deformation and dough (Menjívar, 1990), which gives an idea of the successful processing of bread. Bread dough must be viscoelastic, which means it must have a behavior between viscous liquid and elastic solid. This viscoelastic structure of bread dough provides to retain the gases formed during fermentation.

It is generally accepted that the presence of some of the health-promoting additives such as dietary fibers and antioxidants have significant deteriorative effects on the rheological properties of bread dough due to their influence on gluten network structure (Miś et al., 2017). They exert a thinning and breakage effect on gluten protein fibrils, which lead to premature rupture of gas cells (Han et al., 2019). There are a significant number of reports presenting the various detrimental effects on rheological properties of dough and bread quality as a result of the replacement of wheat flour with various additives that have higher levels of dietary fiber (Gómez et al., 2003; Gül et al., 2009; Almeida et al., 2013; Han et al., 2019; Culetu et al., 2020). However, current studies on the influence of *Opuntia* spp. derivatives on the rheology of bread doughs are limited. The incorporation of *Opuntia* spp. powder lead to an increase in water absorption, compared with the pure wheat flour. An increase in water activity was observed with the increasing amounts of cactus powder in the mixture (Shin & Lee, 2005; Kim et al., 2007; Moreno-Álvarez et al., 2009). These increases could be attributed to the higher dietary fibers content of *Opuntia* derivatives. Hydroxyl groups present in dietary fiber structure in large quantities absorb a significant amount of water by hydrogen bonding with water. Thus, a considerable increase in water absorption of dietary fiber-wheat flour mixture occurs (Rosell et al., 2001; Nawrocka et al., 2016). Dough mixing time also increases with prickly pear powder, but expanding the inclusion level of prickly pear powder into wheat flour decreases this parameter (Shin & Lee, 2005).

The main problem with the addition of cactus powder in the dough mixture is reducing the stability and elasticity. The addition of cactus pear stem flour at concentrations higher than 15% into wheat flour is not recommended due to its weakening effect on dough strength. Similar results were found by Msaddak et al. (2017). They reported a critical reduction in the allographic characteristics such as extensibility and the dough's deformation energy by the replacement of wheat flour (2.5–10%) with *Opuntia ficus-indica* cladodes powder. Shin and Lee (2005) observed similar wheat flour dough stability when prickly pear powder was added at 1.0% level, while a decrease in dough stability increased the prickly pear powder level (2–4%).

The incorporation of dried cladodes (*Opuntia ficus indica* L. Miller) to maize flour causes an increase in dough viscosity that can be attributed to the presence of mucilage, which contributes to the higher water retention, thus provide better quality of the final product (Ramírez-Moreno et al., 2015). During cooling, bread dough viscosity characteristics, particularly pasting temperature, peak viscosity, and setback, showed a higher correlation with kinetic parameters of bread staling (Collar, 2003). Wheat flour retrogradation measured with RVA analysis has been retarded by increasing cactus (*Opuntia humifusa*) powder content (Kim et al., 2007).

Individual addition of *Opuntia* spp. derivatives induced a deleterious effect on dough viscosity. Higher peak viscosity and lower pasting temperatures and setbacks have been described in wheat flour dough containing prickly pear powder at levels of 1%, 2%, 3%, and 4% compared with wheat flour, also increasing trend was observed with the increasing of prickly pear powder. The deteriorative effect of cactus powder has also been seen on dough volume. The addition of powder leads to lower dough volumes than wheat dough (Shin & Lee, 2005). Dietary fibers of *Opuntia* derivatives prevent the formation of complexes between the gluten and starch molecules, which leads to a decrease in the consistency of starch paste, so decreased in pasting temperature and prolongation of the pasting time (Chen et al., 2010).

## 5 Effect of *Opuntia* spp. Derivatives on Physical Properties of Bread

### 5.1 Effect on Loaf Volume

It is complicated to answer the questions “what is good bread” or “how should be a good bread” because quality features of “good bread” change depending on the cultural and regional differences, eating habits, individual experiences, and personal likes and dislikes (Parenti et al., 2020). Besides this broad diversity for good bread, bread quality depends on many factors, mainly qualities and quantities of raw materials, their interactions with each other, and processing techniques (Cauvain, 2012). Despite these factors, the main parameters used in determining the quality of bread can be given as loaf volume, specific volume, crumb characteristics, crumb and crust color, nutritional content, and sensory tests.

*Opuntia* spp. derivatives have various effects on the chemical composition and physical characteristics of bread, depending on their addition level. Table 38.1 shows research studies evaluating the physical properties of bread formulated with a diversity of ingredients derived from *Opuntia* spp. The volume and specific volume of bread is an essential factor in the consumer’s choice. The incorporating of *Opuntia* derivatives in bread formulations significantly reduced the bread’s loaf volume and specific volume depending on the added quantity. When the level of roasted prickly pear seed flour (RPPS) increased above 4% (w/w) to 6%, 8%, and

10% in bread, the final volume of bread was decreased. In comparison, volume and specific bread fortified volume with 2% and 4% RPPS flour showed the highest value with control bread (Ali et al., 2020). Similarly, De Wit et al. (2015) reported a decrease in seed bread volume with the increasing cactus pear (*Opuntia ficus-indica* and *O. robusta*) cladode flour.

Total, insoluble, and soluble dietary fiber fractions of prickly pear may have different effects on a specific volume. The best improving outcome from these three fiber fractions of cactus obtained with soluble fiber addition attributed to mucilage and pectin present insoluble fiber that can improve dough development and gas retention decreasing dough viscosity (Guevara-Arauz et al., 2015). Hence, soluble fiber fraction, which is 20.9% in prickly pear cactus (*Opuntia ficus-indica* cv. Milpa Alta) stems, can be used in bread production without adversely affecting the loaf volume. Recently, some kinds of soluble dietary fibers have significantly improved the viscoelasticity and strength of dough, crumb quality, structural, functional, and in vitro digestion properties, sensory appearance, and exert low glucose release rate (Gan et al., 2020; Huang et al., 2020) of bread. Msaddak et al. (2017) also demonstrated similar conclusions to support these findings, which revealed a significant increase in both volume and specific volume of bread produced from the replacement of wheat flour with 25, 50, 75, and 100 g of cladodes powder (spineless cladodes from *Opuntia ficus-indica* f. *inermis*) per 1 kg wheat flour. *Opuntia ficus-indica* cladodes extract is a good source of phenolics (Msaddak et al., 2017). Therefore, improving effect of cactus soluble dietary fiber fractions may have arisen from both covalent and non-covalent bonds which occur with the interactions between protein and phenolics (Xu et al., 2019), thus a more robust gluten structure is formed and as a result, larger volume bread is obtained. These results are valuable from the technological and economic standpoint because it's difficult to find studies showing the improving effects of fiber-rich ingredients on both volume and specific volume of bread. However, Han and Koh (2011) reported that the addition of phenolic acids adversely affects the dough properties and bread volume in the following order: gallic < syringic < ferulic < caffeic acid. These controversies, regarding the effect of phenolic acids on the bread dough rheology and the quality of bread, may result from the differences in the structure and variety of phenolic compounds and protein, and from other factors such as temperature, pH, and ionic strength of the dough (Özdal et al., 2013; Xu et al., 2019).

It is known that the addition of different dietary fiber sources to bread damages the gluten network, which resulted in lower loaf volume, poor texture, and unsuitable taste and mouthfeel. The adverse effects on loaf volume observed by several researchers with the supplementation of different dietary fiber sources such as banana (*Musa acuminata* X *balbisiana* cv. *Awak*), pseudo-stem flour (Ho et al., 2013), pomegranate seed flour (Gül & Şen, 2017a, b), and the mallow leaves powder (Fakhfakh et al., 2017) in bread formulations.

Reduction of dough and bread quality with the addition of dietary fiber may be connected to competition for water between gluten and dietary fiber fractions, which may cause the redistribution of moisture in the wheat dough. Partial dehydration of gluten adversely affects the viscoelastic network formation because

the protein phase of flour can form gluten if enough water is provided by hydration (Bock & Damodaran, 2013).

## 5.2 Effect on Texture

Textural attributes of bread and loaf volume, are important indicators of bread quality. Still, both of them are easily influenced by factors, such as additives, constituents of flour, and processing conditions. Incorporating new ingredients into a bread formula always brings some technical problems such as low crumb grain and texture as well as an increase in hardness. A similar effect was reported for the addition of *Opuntia ficus-indica* mucilage to wheat flour dough for bread-making, wherein firmness value increased from 6.60 N for wheat flour bread to 9.06 for *O. ficus-indica* mucilage containing bread (Liguori et al., 2020). This agrees with the study of Shin and Lee (2005). They measured a gradual increase in adhesiveness, hardness, chewiness, and gumminess of bread when the amount of substituted *Opuntia ficus-indica* powder increased; the springiness was decreased. Ayadi et al. (2009) tested the effect of spiny (*Opuntia ficus indica* f. *amylocea*) and spineless (*O. ficus indica* f. *inermis*) cladodes flours addition on the textural properties (cohesion, adhesion, stickiness, and hardness) of dough. They observed a significant increase in the adhesion, stickiness, and hardness properties of cake dough. The dough became less elastic and stiff with increase cladodes in the blend. Consequently, cake's hardness at 20% levels of cladodes flours increased from 1.097 to 2.026 N and 1.821 N with spiny and spineless cladodes flours, respectively.

The replacement of *Opuntia robusta* (cultivar "Monterrey") flour with whole wheat flour caused an increase in bread's firmness. Deteriorative effect of *O. robusta* cladodes also determined on the texture of whole carrot cakes, which became stiff and less elastic with increasing cactus flour levels from 0%, 25%, 50%, 75% to 100%. As in bread and cake samples, cactus flour in making biscuits has also increased the hardness values of the biscuits that showed a more compact texture, so more force was required to break them (De Wit et al., 2015).

In general, research shows an increase in the hardness of the bread and other bakery products such as cakes and biscuits when ingredients obtained from *Opuntia* were incorporated. However, Guevara-Arauz et al. (2011) observed a significant differences when they contained nopal (from *Opuntia ficus-indica* cv. *Milpa Alta*) dietary fiber in a tortilla. In this study, the nopal dietary fiber supplemented tortilla was softer (35.68 N) than control tortillas (45.38 N). Moreover, the addition of nopal total fiber, insoluble fiber, and soluble fiber from the same variety decreased both hardness and cohesiveness of fresh bread rolls (Day 0) compared with the control experiment. On the other hand, constant values were measured when total nopal fiber, insoluble fiber, and soluble fiber supplemented bread rolls stored 2 days at room condition (Guevara-Arauz et al., 2015).

Staling rate of breads (supplemented with nopal total fiber, insoluble fiber, and soluble fiber) showed decrease in the following order: control (0.458) > insoluble



fiber (0.381) > total fiber (0.296) > soluble fiber (0.199) after 2 days storage. The low staling rate of soluble dietary fiber-containing bread may be due to the mucilage and pectins' tendency to present insoluble fiber to interact with water (Guevara-Arauz et al., 2015). Soluble fibers (i.e., pectin and galactomannan) have a higher water holding capacity than insoluble fibers (Sivam et al., 2010). Flour from *O. robusta* (cultivar "Monterrey") has a high water holding capacity (De Wit et al., 2015). Higher water retention in maize tortillas with the contribution of cladode (*Opuntia ficus indica* L. Miller) mucilage has been reported (Ramírez-Moreno et al., 2015).

### 5.3 Effect on Color

Crumb and crust colors of the bread are the first essential quality characteristics that are strongly related to marketability and consumer acceptability of the bread and other cereal-based food products. The color that consumers traditionally expect in bread is white or light/dark brown. Besides, the demand for multicolored bakery products is gradually increasing. The incorporation of *Opuntia* spp. ingredients into bread or other bakery products affect the color parameters of these products to varying degrees. When *O. ficus-indica* mucilage is used to replace water to prepare bread from wheat flour, more pronounced effects occur on crumb color than crust color. However, *O. ficus-indica* mucilage's addition does not cause any changes in lightness and redness values of crust, whereas it lowers yellowness of crust (Liguori et al., 2020). In case of addition of cladodes powder (*Opuntia ficus-indica* f. *inermis*, spineless cladodes) to produce flatbreads, lower  $L^*$ ,  $a^*$  and  $b^*$  values for both crust and crumb in comparison to the control were determined (Msaddak et al., 2017). These crusts and crumb color values of fortified bread were decreased with increasing the substitution level of cladodes powder. On the contrary, Shin and Lee (2005) observed the deep red color of crumb increasing gradually with the increasing levels of *Opuntia ficus-indica* powder in bread.

Different dietary fiber fractions of *Opuntia* spp. may have different effects on the crust and crumb color of bread. In this respect, Guevara-Arauz et al. (2015) reported a darker crust and greener crumb coloration due to nopal insoluble fiber (*Opuntia ficus-indica*) addition. Conversely, they did not observe a significant change in either crust or crumb colors when soluble fiber fractions were added.

Kim et al. (2012) noted that cakes' crumb color became darker and more greenish, as cheonnyuncho (*Opuntia humifusa*) powder level increased in the cake flour. Furthermore, the progressive reduction of the lightness and turning from red to blue was remarked on the color of both dried and cooked pasta supplemented with *Opuntia ficus-indica* cladodes extract (Attanzio et al., 2019). Similar findings, decrease on  $L^*$  and  $a^*$  and increase on  $b^*$  value, have also been reported by Dick et al. (2020) when gluten-free crackers were supplemented with *Opuntia monacantha* cladode flour, while they did not found a significant difference in the color parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ) of the cracker containing *Opuntia monacantha* cactus mucilage

powder. It has been stated that the color of tortillas made from commercial maize flour substituted with dried cladodes (*O. ficus indica* L. Miller) changes from the usual white-yellow to green (Ramírez-Moreno et al., 2015). Tendency through the more greenish, higher yellow color of cakes, gluten-free cracker tortillas may be attributed to the chlorophyll present in the flour of *Opuntia* spp.

## 6 Effect of *Opuntia* spp. Derivatives on Nutritive Value of Bread

Bread is one of the foodstuffs with the highest consumption potential in almost all countries. Many consumers' energy needs are met from the bread, which is generally produced from refined wheat flour. The milling process causes a decrease in refined flours' nutritional value compared to that of the whole kernel. To obtain refined wheat flour, outer parts (bran, the **aleurone layer**), which is rich in protein, minerals, and vitamins, and germ of the wheat is separated from the starchy endosperm (Dewettinck et al., 2008). Nutritionally, the concentration of essential nutrients minerals such as Ca, Fe, Zn, folate, vitamin B6, vitamin E and thiamine, as well as some phytochemicals, phenolic compounds, available amino acids, and dietary fiber are significantly higher in the bran fractions and germ than the endosperm (Stevenson et al., 2012). With the increase in consumers' awareness of healthy nutrition, the demand for bread with high nutritional content has increased. In response to consumer demands, fruit and vegetable-based additives in bread formulations have become a novelty. From this point of view, *Opuntia* spp. derivatives are a potential ingredient to improve the nutritional value of bread due to their rich nutrient content.

Effect of the replacement of the refined wheat flour with various amounts of (0%, 2%, 4%, 6%, 8%, and 10%) of roasted prickly pear seed flour on the chemical composition of bread have been studied by Ali et al. (2020). They identified the moisture, fat, ash, dietary fiber, and the baked bread's energy contents ranging from 38.5% to 42.1%, 3.94–5.09%, 0.86–1.89%, 0.82–3.15%, and 406–412 (kcal), respectively. The addition of prickly pear seed flour in bread formulations may significantly increase dietary fiber and ash content of bread. Seeds of the prickly pear can be recognized as a good source for macro and micro minerals (Al-Juhaimi & Özcan, 2013) and dietary fiber (El Kossori et al., 1998). Also, a significant increase in ash (0.44–1.50–3.14–4.78% and 6.42%) and dietary fiber (0.40–2.52–2.60–3.96% and 6.5%) contents were observed in the bread prepared from the mixtures (0–5–10–15% and 20%) of flour from cactus pear (*Opuntia boldinghii* Britton et Rose) stems (cladodes) and wheat flour (Moreno-Álvarez et al., 2009). In general, health benefits of dietary fibers such as reduce the risk of cardiovascular disease, colon cancer, chronic constipation, diverticular disease and haemorrhoids, type 2 diabetes modulates the glucose and lipid metabolisms, and lower body weights have been reported (Sáenz, 1997; Dahl & Stewart, 2015). Therefore, cladodes flour from various *Opuntia* species can be suggested as an

excellent potential source of dietary fiber (Sáenz, 1997; Arias-Rico et al., 2020) and can be used to increase the dietary fibre content of bread and other bakery products.

The protein content of bread from wheat flour should be at least 10% based on a dry matter basis (Anonymous, 2013). From these respect, flour mixtures composed of wheat flour and stems (cladodes) flour of cactus pear (*Opuntia boldinghii* Britton et Rose) (CF) in the range of 0%, 5%, 10%, 15%, and 20% levels meet this requirement with the protein content of 16.4%, 16.1%, 15.2%, 14.9%, and 13.7%, respectively. Moreover, it can be remarked that the protein content of bread should not be less than 13% (Granito & Guerra, 1995); hence the substitution of cladodes powder into bread provides a sufficient (>13%) protein content (Moreno-Álvarez et al., 2009).

A comparable trend was also reported (Liguori et al., 2020) for total phenolics (from 7.92 to 54.72 mg GAE 100 g<sup>-1</sup> DW), antioxidant activity by ABTS (from 50.4 to 83.6 μmol TE 100 g<sup>-1</sup> DW), and FRAP (from 269 to 626 μmol TE 100 g<sup>-1</sup> DW) assays in baked bread after addition of fresh *O. ficus-indica* mucilage (in substitution to water) compared with control bread (without any supplement). Similarly, Msaddak et al. (2017) reported that cladodes powder obtained from *Opuntia ficus-indica* f. *inermis* (spineless cladodes) supplementation in flatbread enhanced their total phenolics content, DPPH radical scavenging activity, Fe<sup>2+</sup> chelating activity, and Fe<sup>3+</sup> reducing power.

It is important to remark that apart from bread, *Opuntia* seed powders may enhance the fiber, phenolics, flavonoid contents, and antioxidant activity of rice-based extrudates (Rayan et al., 2018). *Opuntia* cladode extract can be suggested to fortify pasta because of its blood cholesterol- and glucose-lowering capabilities (Attanzio et al., 2019). The incorporation of cactus pear (*Opuntia ficus-indica* L. Mill, and *Opuntia robusta* Wendl) cladode flour increased the fiber content of crunchy oats biscuits. In contrast, cakes' fibre content prepared from blends containing 0%, 25%, 50%, 75%, and 100% of cladode flour remained more or less the same in all samples (De Wit et al., 2015). The incorporation of nopal (*Opuntia ficus-indica* cv. *Milpa Alta*) allows an increase in the dietary fiber, polyphenols, Trolox equivalent antioxidant capacity, and vitamin C as well as lowers levels of glucose (4.43 mmol/L), total cholesterol (4.27 mmol/L), LDL (1.96 mmol/L) and triglycerides content in tortillas (Guevara-Arauz et al., 2011). Cactus pear peel and alcohol-insoluble solids powder from *Opuntia ficus-indica* is another alternative as a natural additive or substituted material with higher nutritional components, sugar compounds and phenolic compounds in the production of wheat flour biscuits (El-Shahat et al., 2019).

## 7 Effect of *Opuntia* spp. Derivatives on Sensory Properties of Bread

Based on several studies, bread enriched with *Opuntia* spp. derivatives are valuable from the nutritional point of view and can provide important health benefits. However, if these bread's organoleptic properties do not meet the consumers'

expectations, it will be difficult for them to become a commercial product. Therefore, many researchers have been focusing their interest on the sensory properties and consumer acceptance of *Opuntia* enriched bread and/or other bakery products.

Bread contained 3% cactus chounnyuncho (*Opuntia humifusa*) powder has been reported as the most preferred bread according to the sensory test (Kim et al., 2007). Following the same trend, Moreno-Álvarez et al. (2009) showed that bread formulated with 5% and 10% cactus pear (*Opuntia boldinghii Britton et Rose*) stems (cladodes) flour were most accepted in terms of flavour, odor, color, and texture compared with bread formulated with pure wheat flour (control) and 15% and 20% cactus pear stem flour. The highest texture score was recorded for bread supplemented with 5% cactus pear stem flour. The texture of bread that feels by touch or in the mouth is a decisive criterion for consumers' acceptance or rejection. About 20% of bread's acceptability is related to its textural properties (Lassoued et al., 2008). Indeed, textural hardness and other sensory attributes as color, odor, taste, and overall acceptability of the bread prepared from *Opuntia ficus-indica* cladodes powder, remained acceptable up to 7.5% substitution level. In comparison, bread texture became unacceptable when the substitution level increased to 10%, and this result was confirmed with the allographic tests (Msaddak et al., 2017). Moreover, the sensory evaluation of wheat flour bread enriched with 2.0% *Opuntia ficus-indica* powder has higher sensorial scores than wheat flour bread (Shin & Lee, 2005).

Addition of roasted *Opuntia ficus-indica* seed flour resulting in acceptable changes in the sensory quality characteristics of the bread at low levels of up to 6% supplementation, but increasing the supplementation level of seed flour more than 6% (8% and 10%) makes bread unacceptable (Ali et al., 2020). Guevara-Arauz et al. (2015) reported similar observations in their evaluation of bread rolls' sensory characteristics added with total fiber and insoluble fiber from nopal flour (*Opuntia ficus-indica* cv. *Milpa Alta*). On the contrary, the addition of soluble fiber to bread rolls resulted in significant improvements on the sensory features (surface characteristics, crust color, crumb color, taste mouthfeel texture overall quality) of rolls and other bread quality characteristics.

Regarding the sensory analysis, high concentrations of fibre results in unfavorable sensory features of the bread. For example, Lu et al. (2017) mentioned that the sensory properties of bread decreased when the concentration of both apple pomace and skimmed apple pomace exceeds 3%. Dried peel (7.5%) or alcohol-insoluble solids (7.5%) from *Opuntia ficus-indica* can be used to substitute wheat flour biscuits in terms of sensory characteristics such as; appearance, color, taste, flavor and overall acceptability (El-Shahat et al., 2019). However, increasing their substitution level to 10% makes biscuits unacceptable because of the high fiber content. Cladodes flour from *Opuntia ficus indica* has significant effects on cakes' organoleptic characteristics (Ayadi et al., 2009) as those on biscuits. Indeed, with the increase of cladodes flours levels (from 0% to 5%, 10%, 15%, and 20%) in cake formulation, total sensory scores decreased. Therefore, good quality cakes can be prepared when cladodes flours are used up to 5% substitution level. On the other hand, in the literature (Gomes et al., 2016), no loss was observed on the acceptance

(flavor, texture, and overall impression) or shelf life of bread using green banana (with its peel) flour at higher concentrations (up to 20%). The remarkable point in this research that, despite the lower physical and chemical quality attributes of bread supplemented with green banana flour, that did not affect the consumer's desire to consume the products. Bread enriched with fresh *O. ficus-indica* mucilage (in substitution to water) has received general appreciation from the judges (Liguori et al., 2020). Therefore, fresh cactus mucilage can be used as an alternative ingredient to increase the antioxidant intake in bread production without losing its sensory attributes.

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# Chapter 39

## *Opuntia* spp. Products and By-products as a Potential Source of Edible Films and Coatings



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**Abstract** *Opuntia* spp. is the main genus of the *Cactaceae* family that includes about 1500 species in the world. *Opuntia* spp. edible parts include cladodes and their fruits, and their by-products are rich in dietary fiber, minerals, and bioactive compounds. These ingredients are currently being investigated for their application in edible films and coatings. Edible films and coatings are defined as continuous matrices of polymeric material. Among various functions and specific characteristics of edible films or coatings are; suitable water barrier efficiency, control of the gas exchange, retard solute, oil, and fat migration. Edible films and coatings research from *Opuntia* polysaccharides and its derivatives are an exciting and novelty area that has been developed in recent years. This chapter aims to evaluate the state of *Opuntia*'s art as a source of polymers to incorporate as edible films and coatings.

**Keywords** Pectin · Cellulose · Peel · Mucilage · Environment

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## 1 Introduction

Consumers currently demand food of higher nutritional quality in combination with packaging that is friendly to the environment. This market trend has prompted researchers to develop natural and biodegradable packaging (Dias et al., 2010; Pelissari et al., 2013; Dhumal & Sarkar, 2018; Gheribi & Khwaldia, 2019). Furthermore, the food industry's main target is to maintain the quality and wholesomeness of their products for consumer acceptance (Kumar & Neeraj, 2019). All packaging designed for food must satisfy the primary purpose of extending the food shelf life as much as possible. Thereby preserving food from microbial deterioration, oxidation, color, and flavor changes (Khazaei et al., 2016).

There is an increasing awareness that conventional packaging, especially plastic products, creates extensive environmental damage to water resources and the entire ecosystem (Nešić et al., 2019). As is well known, the packaging is an essential part of the food industry; however, more than 90% of all plastic materials are non-biodegradable and are derived from non-renewable sources. On the other side, lower than 5% of the plastic products are recycled (Hammam, 2019; Beikzadeh et al., 2020).

Science has the challenge of implementing innovative technologies to develop eco-friendly packaging that achieves food safety, quality, and freshness. Ideal candidates to replace conventional polymers are renewable and biodegradable edible films or coatings. It can be designed from various materials such as proteins, carbohydrates, lipids, or combinations (Krochta, 1997; Daudt et al., 2016; Vargas et al., 2017). They provide protection against microbial contamination and extend food shelf life (Salehi, 2019; Kumar & Neeraj, 2019). This technology is suitable for human consumption and even if they are unconsumed they could be biodegraded efficiently (Vásquez et al., 2009; Kadzińska et al., 2019). Edible films and coatings selection consider various factors like cost, availability, functional attributes, mechanical (tension and flexibility), and optical (brightness and opacity) properties. Barrier effect against the flow of gases, resistance to water, microbial growth, sensory acceptability, are among others. Nowadays, edible films or coatings have been developed from various biopolymers extracted from natural sources.

*Opuntia* ssp. is the main genus of the *Cactaceae* family that includes about 1500 species (Pimienta-Barrios et al., 2008; Osorio-Esquivel et al., 2012). The native plant is widespread in America; however, it has been introduced in other parts of the world, such as Europe and Africa (Amamou et al., 2020). Mexico and Italy are the most important producers of *Opuntia* ssp. (Mondragon-Jacobo, 2020). *Opuntia* ssp. edible parts include cladodes and their fruits. Various food products have been developed from *Opuntia* ssp., such as juices and jams, powders, and drinks (Ayadi et al., 2009; Abdel-Hameed et al., 2014). Likewise, functional food have been designed with specific components extracted from cladodes or fruits. Moreover, the aforementioned product's process generates by-products, around 20% and 45% of cladodes and fruits, respectively (Nassar, 2008). These by-products are rich in dietary fiber, minerals, and bioactive compounds (Bensadón et al., 2010). These

ingredients are currently used to develop functional food and being investigated for their application in edible films and coatings (González Sandoval et al., 2019). This chapter will address the development of edible films and coatings, especially those developed from chemical components extracted from *Opuntia* spp. as well as the products and by-products obtained from its processing.

## 2 Edible Films

According to Jeya Jeevahan et al. (2020), edible packaging is a thin layer either formed directly on the food surface or separately as a sheet/film. When wrapped over the food surface, it is called an edible coating, and the latter is an edible film. Even though both terms are used together as a phrase. The key difference between them is their preparation and application process (Pop et al., 2020). Coatings are applied in liquid form, so food is dipped into a polymer solution. Simultaneously, films are obtained as sheets and then used as laminating, wrapping, and packaging (Ramos-García et al., 2010; Dhumal & Sarkar, 2018; Kadzińska et al., 2019).

Materials used for edible films or coatings production are from food-grade biopolymers. Biopolymers have been divided into three categories: polysaccharides (starch and its derivatives, alginates, chitosan, cellulose and derivatives, and pectin), lipids (waxes and paraffin, acetyloglyceridy, and shellac resins) and proteins (casein, gelatin, whey proteins, and soy proteins) (McHugh & Avena-Bustillos, 2012; Dhumal & Sarkar, 2018; Hammam, 2019).

Among various functions and specific characteristics of edible films or coatings are; suitable water barrier efficiency, control of the gas exchange, retard solute, oil, and fat migration. Retard organic vapor transfers and improve food mechanical properties to facilitate handling and carriage. Sensorial characteristics such as color, shininess, transparency, roughness, or sticking can be improved (Umaraw & Verma, 2015). However, the most significant ones are physical and food properties protection, additives carriers, and shelf life extension (Pop et al., 2020).

Polysaccharides are the most extensively used biopolymers for edible films production. They are expected to be a good gas barrier due to their ability to form a cross-linking network between polymer chains by hydrogen bonds. It has been reported that polysaccharides are colorless, tasteless, odorless, and non-toxic. Furthermore, they can inhibit bacterial growth due to water activity reduction. However, they show reduced resistance to water vapor and limited mechanical strength. Films produced from proteins also demonstrated lower resistance to water vapor but used to decrease moisture loss, limit oxygen permeability, and reduce fat loss. They improve mechanical and sensory properties, increasing nutritional food value, and maintain consistent degradability with environmental demands. Proteins also can carry antimicrobial substances, flavors, and colors that improve the functionality of packaging materials. Lipids show good resistance to water vapor, especially in fresh products but cannot establish self-supporting structures. These films are misty, brittle, unsteady, and easily they leave a lipid taste. These characteristics

affect food sensory properties and, thus, reduce their marketing, so they are unsuitable for producing edible films. Lipids are, therefore, used for coating applications or as an additive along with polysaccharide and/or protein to produce composite films (Velickova et al., 2013; Hammam, 2019; Jeya Jeevahan et al., 2020).

As mentioned above, films made from proteins or polysaccharides are known for their suitable mechanical properties. However, they are moisture permeable. Simultaneously, lipid films (wax, fats, and oils) exhibit less water vapor permeability. However, their mechanical resistance is weak and highly oxygen permeable. Since they do not satisfy the specifications of an ideal packaging film, a composite film is the best way out of it including combination of various films to improve their permeability or mechanical properties (Bourtoom, 2008; Kadzińska et al., 2019; Hammam, 2019).

Umaraw and Verma (2015) mentioned that blending polysaccharides, proteins, and/or lipids could form composite films by extruding, laminating, or emulsion forming two or more polymers. Films with suitable mechanical properties are produced from proteins or polysaccharides. However, they are moisture permeable. It depends on the specific application to improve some mechanical properties or gas permeability affecting film barrier properties. Composite film functional properties not only depend on individual film characteristics, but also the interaction among their constituents. Some studies involved protein-protein, carbohydrate-carbohydrate, protein-carbohydrate, and lipid-based edible films and ternary and quadruple edible films and coatings (Dhumal & Sarkar, 2018).

Two standard techniques are employed for edible film preparation, wet (solvent casting), and drying (compression molding or extrusion) technique. The wet processing method is produced from materials with film-forming ability. Film solution is dispersed and dissolved mainly in water (alcohol or their mixture). Some additives are incorporated (plasticizers, bioactive molecules, color, and flavor compounds). In some cases, polymer dispersion can be adjusted by modifying pH or temperature. The solution is then cast and dried at specific conditions (temperature and relative humidity) to achieve finished edible films. Dipping, spraying, brushing, and panning methods are used when they are applied as coatings onto the food surface. Otherwise, some polymers thermoplastics features like compression, molding, or extrusion are applied in the drying process (Umaraw & Verma, 2015; Dhumal & Sarkar, 2018).

A suitable edible film needs to be healthy, safe, have excellent sensory quality, high barrier properties, high mechanical strength, high microbial stability, simple to produce, non-polluting, and low cost (Jeya Jeevahan et al., 2020). It must also be compatible with the food to be applied. Several studies have been conducted on the application of multiple biopolymers using composite edible films and coatings, mainly on fruits and vegetables and dairy, meat, and seafood products. The effectiveness of a particular composite film or coating depends on selecting the individual biopolymer and the compatibility between them. Some researchers have recently incorporated vitamins, flavors, probiotics, and nutritional supplements into edible films (Dhumal & Sarkar, 2018; Hammam, 2019; Kumar et al., 2020; Pop et al., 2020).

García et al. (2000) reported that film's key physical characteristics depend on various factors like its molecular integrity, the relationship between amorphous and crystalline zones, polymer chains' mobility, and hydrophobic and hydrophilic zones that they form. Additional research considers parameters as film formulation, forming technic, and polymer characteristics, solvent, and plasticizer (Han et al., 2006; Akbari et al., 2007; Sánchez-González et al., 2010).

### 3 *Opuntia* Polysaccharides and Other Chemical Compounds as a Source of Polymers for Edible Films and Coatings

Nopal is the most common vegetable from the genus *Opuntia*. Cladodes form nopal, or stems, cactus pads, cactus vegetables, phylloclades, nopales, or pencas, covered with spines (Angulo-Bejarano & Paredes-López, 2012). Predominantly, there are two edible products derived from species of the genus *Opuntia*; cladodes, tender part of the cactus stem, and prickly pear fruits or also called cactus pear or xoconostle, a sweet pulp. Cactus pear fruit consists of peel, seeds, and pulp (Hernández-Fuentes et al., 2015; Arias-Rico et al., 2020). *Opuntia* peel makes up 40–60% of the whole fruit, generally discarded. Even so, fruit peel may constitute an excellent source of compounds with technological importance (Ramadan & Mörsel, 2003; Hernández-Carranza et al., 2019).

*Opuntia* plants are principally composed of protein, carbohydrates, lipids, and fiber. Other trace compounds include pigments (chlorophylls, and betalain), vitamins (C, and E), phenolic compounds (Barba et al., 2017; Cruz-Bravo et al., 2019) are shown in Table 39.1. However, the main compounds for edible films and coatings manufacture are carbohydrates (starch, and pectin), proteins, and lipids (waxes) (Hall, 2012; Pérez-Gago, 2012; Soliva-Fortuny et al., 2012). Cladodes and prickly pear chemical composition are highly influenced by ripening stage, species type, post-harvest treatment, environmental conditions, and plant age (Guevara-Figueroa et al., 2010; Contreras-Padilla et al., 2011; Astello-García et al., 2015; Salehi et al., 2019).

#### 3.1 Polysaccharides

For edible coatings, polysaccharides are the most widely material used for food encapsulation; some typical applications are:

1. Starch and derivates: amylose, amylopectin, dextrans, maltodextrins, polydextrose, syrups, and cellulose and their derivatives.
2. Plant exudates and extracts: Gum Arabic, guar gum, and pectin (Nedovic et al., 2011).

**Table 39.1** *Opuntia* species proximal chemical composition

Specie	Cladode type	Proteins	Lipids	Carbohydrates	Starch	Fiber	Ash	Reference		
<i>O. ficus-indica</i> f. <i>amyloceae</i>	Spiny cladodes <sup>a</sup>	8.74	3.95	60.36	7.63	51.24%	25.65	Ayadi et al. (2009)		
	Spineless cladodes <sup>a</sup>	8.88	4.69	60.93	13.09	41.83%	23.3			
<i>O. ficus-indica</i>	Cladodes <sup>a</sup>	3.7–9.4	1.0–1.3	ND	ND	ND	14.2–21.0	Salehi et al. (2019)		
<i>Opuntia</i> spp. var. Blanco	Cladodes <sup>b</sup>	6.7	0.1	61.4	ND	15.0	17.3	Guevara-Figueroa et al. (2010)		
<i>Opuntia</i> spp. var. Manso		16.0	0.1	55.1	ND	10.8	18.8			
<i>Opuntia</i> spp. var. Amarillo		15.1	0.6	63.2	ND	6.2	14.9			
<i>Opuntia</i> spp. var. Cristalino		9.4	1.5	66.5	ND	7.7	14.8			
<i>Opuntia</i> spp. var. Duraznillo		13.5	1.1	69.8	ND	7.1	19.7			
<i>Opuntia</i> spp. var. Morado		13.9	ND	80.9	ND	ND	5.2			
<i>Opuntia</i> spp. var. Tapon-I		15.1	ND	66.0	ND	ND	18.9			
<i>Opuntia</i> spp. var. Tapon-II		17.4	1.8	42.4	ND	20.4	19.5			
<i>Opuntia</i> spp. var. Tempranillo		13.4	ND	61.9	ND	5.5	19.3			
<i>Opuntia</i> spp. var. Tablets		4.2	0	6.7	ND	51.6	37.3			
<i>Opuntia streptacantha</i>		Cladodes <sup>c</sup>	11.2	0.73	ND	ND	7.3		12.6	Santos-Díaz et al. (2017)
<i>Opuntia hyptiacantha</i>			11.0	0.80	ND	ND	6.5		15.1	
<i>Opuntia megacantha</i>	10.7		0.69	ND	ND	6.5	13.6			
<i>Opuntia albicarpa</i>	11.6		0.75	ND	ND	6.5	13.2			
<i>Opuntia ficus-indica</i>	11.2		0.69	ND	ND	5.9	14.4			
<i>Opuntia humifusa</i>	4.7		1.25	ND	ND	50.3	2.0			
<i>Opuntia ficus indica</i> var. <i>Atlixco</i>	Cladodes <sup>a</sup>	1.13	1.22	ND	ND	64.25	16.54	Bensadón et al. (2010)		

(continued)



**Table 39.1** (continued)

Specie	Cladode type	Proteins	Lipids	Carbohydrates	Starch	Fiber	Ash	Reference
<i>Opuntia ficus-indica</i> var. <i>Milpa Alta</i>	Cladodes <sup>a</sup>	1.14	1.42	ND	ND	62.05	16.29	Bensadón et al. (2010)
<i>Opuntia</i> spp.	Cladodes <sup>a</sup>	0.73	0.51	9.57	ND	3.60	1.64	Barba et al. (2017)
<i>Opuntia ficus-indica</i>	Prickly pear fruit (pulp) <sup>b</sup>	5.13	0.97	58.3	4.55	20.5	8.50	El Kossori et al. (1998)
	Prickly pear fruit (skin) <sup>b</sup>	8.30	2.43	27.6	7.12	40.8	12.1	
<i>O. ficus-indica</i>	Pear peel <sup>c</sup>	3.72	1.44	85.79	ND	ND	9.05	El-Shahat et al. (2019)
<i>O. xocostole</i> F.A.C. Weber cv. <i>Cuaresmeño</i>	Pear fruit <sup>a</sup>	4.0	2.5	9.8	ND	30.8	11.3	Arias-Rico et al. (2020)
<i>O. matudae</i> Scheinvar cv. <i>Rosa</i>	Pear fruit <sup>a</sup>	4.8	11.9	29.9	ND	36.8	11.7	
<i>O. ficus-indica</i>	Pear fruit (Green tuna) <sup>a</sup>	Traces	1.14	ND	ND	42.93	17.07	Bensadón et al. (2010)
	Pear fruit (Red tuna) <sup>a</sup>	Traces	0.95	ND	ND	27.51	13.14	
<i>O. ficus-indica</i>	Pear fruit <sup>c</sup>	0.21–1.60	0.09–0.70	12–17	ND	0.02–3–15	0.3–1.0	Salehi et al. (2019)
<i>O. ficus-indica</i> var. <i>Gialla</i>	Pear fruit <sup>d</sup>	0.52	0.037	15.68	ND	ND	0.348	Melgar et al. (2017)
<i>O. ficus-indica</i> var. <i>sanguigna</i>		0.84	0.063	18.0	ND	ND	0.42	
<i>O. engelmannii</i>		1.62	0.38	33.0	ND	ND	0.75	
<i>O. ficus-indica</i> L.	Seeds <sup>b</sup>	4.78	5.0	76.48	ND	12.47	1.27	Özcan and Al Juhaimi (2011)

(continued)

**Table 39.1** (continued)

Specie	Cladode type	Proteins	Lipids	Carbohydrates	Starch	Fiber	Ash	Reference
<i>O ficus-indica</i> sp.	Seeds <sup>c</sup>	11.8	6.77	1.59	5.35	54.2	5.90	El Kossori et al. (1998)

ND not determined

<sup>a</sup>Results expressed as g/100 g dry matter

<sup>b</sup>Results expressed as % dry matter

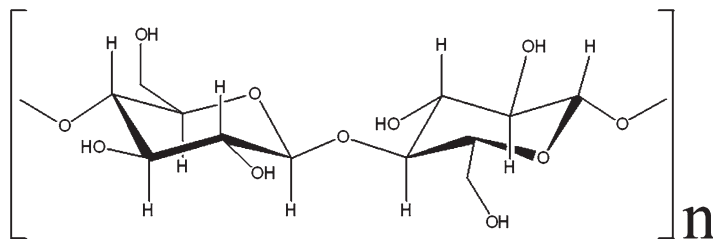
<sup>c</sup>Results expressed as percentage (%)

<sup>d</sup>Results expressed as g/100 g fresh weight

El Kossori et al. (1998) reported that the content of hemicellulose, cellulose, pectin, and lignin in *Opuntia ficus-indica* was different for each section of prickly pear fruit (pulp, skin, and seeds). Pectin was the most abundant polysaccharide present in pulp (70.3% of total fiber), whereas cellulose was in higher concentration in the skin (71.4% of total fiber) and seeds (83.2% of total fiber). On the contrary, the most lower content reported was for lignin: 0.01%, 0.06%, and 0.19% in pulp, skin, and seeds, respectively. Amamou et al. (2020) evaluated the influence of pH (acidic, neutral, or alkaline medium) on the extraction of polysaccharides from *Opuntia macrorhiza* fruit peels. The authors reported that the acidic medium led to the highest polysaccharides yield (13.7% dry matter) and uronic acid content (50.5%) concerning the neutral and alkaline medium. Overall, coatings made from polysaccharides, like cellulose, pectin, starch, alginates, are good barriers, but their hydrophilic nature constitutes poor barriers to moisture (Perera, 2007).

### 3.1.1 Mucilage

Mucilage is a water-soluble polysaccharide, which has been widely applied to develop films. Gheribi et al. (2019b) extracted mucilage from prickly pear peels from *O. ficus-indica* species, representing 3.08% (dry weight). Peel's mucilage was constituted by carbohydrates (97%), such as galactose (54%) and arabinose (34%), with a reduced amount of xylose (10%), and galacturonic acid (9.1%). Besides, mucilage extracted from prickly pear peels exhibited promising functional properties including emulsifier, foaming agent, or water holder. Similarly, Habibi et al. (2004) reported that the most abundant sugar of mucilage (*O. ficus-indica*) was arabinose (32.7%), galactose (23.5%), and galacturonic acid (14.2%). The presence of galactose and arabinose suggested arabinogalactan, which consisted of a chain of (1 → 4)-linked β-D-galactopyranosyl residues (Fig. 39.1). Majdoub et al. (2001) reported that pectin cladodes aqueous extracts and pear peels were predominately composed of galacturonic acid and rhamnose (Table 39.2). *O. ficus-indica* mucilage extracted at pH between 4 and 8 with and without calcium could form edible films (Espino-Díaz et al., 2010).



**Fig. 39.1** Chemical structure of mucilage from *Opuntia* spp.

### 3.1.2 Pectin

Pectin belongs to the heteropolysaccharides family (Fig. 39.2), and they are composed of anionic D-galacturonic acid units in an  $\alpha$ -(1  $\rightarrow$  4) chain, which are interrupted occasionally by  $\alpha$ -(1  $\rightarrow$  2)-rhamnose residue (Maxwell et al., 2012; Salehi et al., 2019). It is constituted by different monosaccharides, like rhamnose, arabinose, galactose, among others (Naqash et al., 2017). It performs a significant role as a structural element of the plant cell wall (Atmodjo et al., 2013; Dranca & Oroian, 2018). Chain length and esterification degree affect its solubility, viscosity, and gelation properties. Esterification degrees above 50% are labeled high-methoxyl, and below 50% as low-methoxyl pectin (Hall, 2012).

Some studies reported the presence of pectin in *Opuntia* species (Cárdenas et al., 2008). An alkaline process extracts pectin from cactus with calcium as a sequestering agent. Uronic acids (85.4%), galactose (7.0%), arabinose (6.0%), and minor quantities of rhamnose and xylose are the main pectin components. Lira-Ortiz et al. (2014) evaluated the extraction of low methoxyl pectin from prickly pear fruit (*Opuntia albicarpa* Scheinvar 'Reyna'). The extracted polysaccharides from prickly pear peel fruit were high-molecular-weight pectin. Molecular weight-average was near  $10.16 \times 10^5$  g/mol. High molecular weight is associated with high viscosities and shear thinning behavior in aqueous dispersions.

### 3.1.3 Cellulose

It is the most abundant polysaccharide worldwide. Cellulose (Fig. 39.3) is the major component of plant cell walls, and it is composed of repeating glucose units in  $\beta$ -1,4-linkage. Cellulose is an isotactic  $\beta$ -1,4-polyacetal of 4-O- $\beta$ -D-glucopyranosyl--D-glucose (Coffey et al., 2006; Baldwin, 2020). Unfortunately, cellulose, due to its chemical composition, is not soluble in water. Carboxymethyl cellulose is a polysaccharide prepared by carboxymethylation of cellulose. Besides its biodegradability, it is water-soluble and non-toxic. It produces transparent films. However, edible films based only on this polymer are limited due to their low water barrier properties resulting from its hydrophilic nature. The blending of carboxymethyl cellulose with

**Table 39.2** Principal polysaccharides extracted from *Opuntia* species

Part of plant	Arabinose	Rhamnose	Xylose	Mannose	Galactose	Glucose	Galacturonic acid	Reference
Cladode <sup>a</sup>	15.0	46.0	9.1	4.1	11.0	1.9	10.2	Majdoub et al. (2001)
Pear peel <sup>a</sup>	0	48.6	0	0	0	0	51.8	Majdoub et al. (2001)
Cladode mucilage <sup>a</sup>	5.32	ND	3.27	ND	5.71	23.29	0.85	Espino-Díaz et al. (2010)
<i>O. ficus-indica</i> (pulp)	ND	ND	0	0	ND	35.0	ND	El Kossori et al. (1998)
<i>O. ficus-indica</i> (Pear peel)	ND	0.069%	0.552%	0.07%	0.073%	4.466%	ND	El-Shahat et al. (2019)
<i>O. ficus-indica</i> (mucilage gum)	44.04%	7.02%	22.13%	ND	20.43%	ND	6.38%	Medina-Torres et al. (2000)
<i>O. dillenii</i> (Ker-Gawl) (mucilage powder)	38.80%	15.70%	5.10%	ND	33.0%	5.10%	2.50%	Kalegowda et al. (2017)

ND not determined  
<sup>a</sup>wt% of dry compound

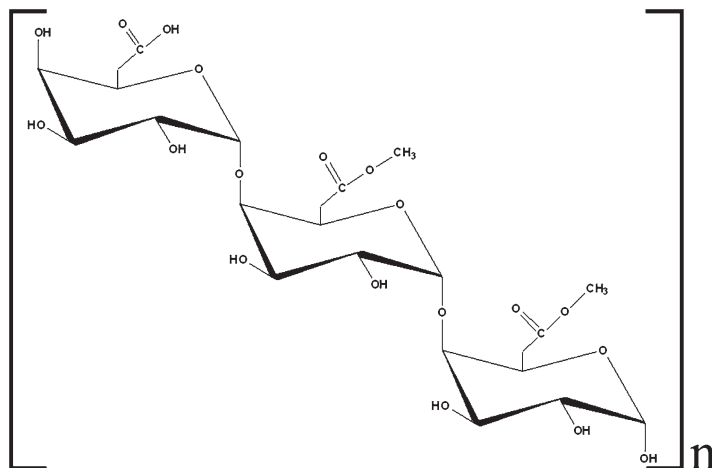
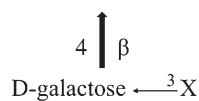
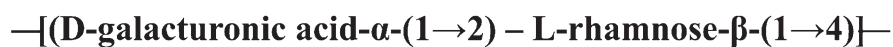


Fig. 39.2 Chemical structure of pectin



Where X = arabinose or xylose

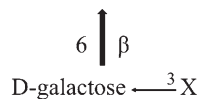
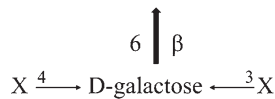


Fig. 39.3 Chemical structure of cellulose. (Adapted from Fox, 2011)

other biodegradable polymers like chitosan, starch, and collagen was reported to improve their inherent shortcomings (Salama et al., 2019).

### 3.2 Proteins and Lipids

It has been proposed that proteins have good film-forming abilities similar to polysaccharides. Protein films have good oxygen barrier properties, and they are an excellent option to use for high-fat food (Candoğan et al., 2017). Furthermore,

lipids for edible films include fatty acids and fatty alcohols, waxes (beeswax, carnauba wax, candellia wax), glycerides, and phospholipids. Lipid-based films provide a good moisture barrier due to their hydrophobic properties, reducing moisture loss (Nedovic et al., 2011; Candoğan et al., 2017).

Ramadan and Mörsel (2003) reported 36.8 g/kg on the dry weight of total lipids extracted from the peel. Six different cultivars of fruits and cladodes of prickly pear were analyzed (Andreu-Coll et al., 2019). Fruits and cladodes showed a high content of lipids, saturated, monounsaturated, and polyunsaturated fatty acids. However, no reports about *Opuntia* proteins or lipids extraction or edible film applications are reported, probably due to their low concentration.

#### 4 *Opuntia* and Its Derivates Edible Films and Coatings Applications

Edible films and coatings research from *Opuntia* polysaccharides and its derivates are an exciting and novelty area that has been developed in recent years. Mucilage from cladodes or other plant parts and by-products from fruits (prickly pear peel) are the main sources of polymers as a major matrix formation component. Valuable research regarding *Opuntia* applications to edible films and coatings are listed in Table 39.3.

Regarding inedible mucilage films, Espino-Díaz et al. (2010) managed to develop with *O. ficus-indica*, testing different pH values, calcium concentration, and different types of plasticizers. They found that both calcium content and pH affect film mechanical properties and water vapor permeability (WVP). Calcium helps to form intramolecular bonds with carboxylic groups by modifying WVP as well as breaking force. These types of hydrophilic films with calcium improve WVP and tensile strength properties. Dominguez-Martinez et al. (2017) developed composite edible films using mixtures of mucilage, chitosan, and polyvinyl alcohol (PVA). The combination of this type of material increases WVP. These types of films were stable in the polymeric matrix formation with suitable mechanical properties. The combination of PVA with mucilage reinforces mechanical properties, and they can be applied for coating fruits that are considered fragile.

De Campo et al. (2018) designed nanocapsules of *O. monacantha* mucilage and zeaxanthin extract. Zeaxanthin is a carotenoid isomer of lutein, which is extremely sensitive to UV light and heat. These capsules could protect against zeaxanthin degradation. Also, mucilage is composed of anionic polysaccharides negatively charged, which allows the formation of stable intra and intermolecular bonds when a positive cation such as calcium or magnesium is added. The results obtained suggest that retention of zeaxanthin depends on the storage temperature. Gheribi et al. (2018) evaluated the use of different polyols plasticizers to elaborate cladode mucilage edible films. Polyethylene glycol and sorbitol were the suitable compounds that provide the most desirable mechanical properties. They discovered that mucilage

**Table 39.3** *Opuntia* spp. edible films and coatings applications

Film/coating	<i>Opuntia</i> specie	Results	Reference
Coatings on strawberries	<i>O. ficus-indica</i>	Mucilage edible coatings, extension of shelf life, improves sensory properties	Del-Valle et al. (2005)
Films	<i>O. ficus-indica</i>	Mucilage edible films with calcium, low WVP, and high force values	Espino-Díaz et al. (2010)
Coatings on fresh-cut red delicious apples	<i>O. ficus-indica</i>	Inhibition of enzymatic browning, an increase of shelf life, and fruit firmness	Zambrano-Zaragoza et al. (2014)
Coatings on kiwifruit slices	<i>O. ficus-indica</i>	Maintain firmness, visual quality, and reduced weight loss	Allegra et al. (2016)
Coatings on 'Dottato' fig ( <i>Ficus carica</i> L.) fruit	<i>O. ficus-indica</i>	Antibacterial coating, an increase of shelf life, and carotenoids	Allegra et al. (2017)
Films	<i>O. tomentosa</i>	Strong films in combination with chitosan and polyvinyl alcohol	Dominguez-Martinez et al. (2017)
Coatings on fresh-cut pineapple	<i>O. ficus-indica</i>	Mucilage, chitosan, pullulan, and linseed coatings. Low WVP, antibacterial properties, suitable mechanical barrier	Treviño-Garza et al. (2017)
Films	<i>O. ficus-indica</i>	Prickly pear by-products with CMC, antioxidant edible films	Aparicio-Fernández et al. (2018)
Nanofilms	<i>O. monacantha</i>	Mucilage and zeaxanthin extract, protection against oxidation	de Campo et al. (2018)
Films	<i>O. ficus-indica</i>	Mucilage with different polyols as plasticizers, suitable film-forming	Gheribi et al. (2018)
Films	<i>O. ficus-indica</i>	Combination of mucilage and pectin, enhancing elongation and tensile strength	Guadarrama-Lezama et al. (2018)
Films	<i>O. oligacantha</i>	Combination of starch and bio extracts of peel, antioxidant edible films	Cenobio-Galindo et al. (2019)
Coatings on fresh-cut potatoes	<i>O. dillenii</i>	Inhibition of enzymatic browning, an increase of shelf life, and fruit firmness	Wu (2019)
Films	<i>O. oligacantha</i>	Inhibition of UV-C light, mechanical resistance improved	Gheribi et al. (2019a, b)
Films	<i>O. ficus-indica</i>	Prickly pear edible films, pseudoplastic behavior	Gheribi et al. (2019a, b)
Films	<i>O. ficus-indica</i>	Different varieties of <i>O. ficus</i> , capena variety with low permeability	Sandoval et al. (2019)
Films	<i>O. ficus-indica</i>	Prickly pear by-products edible films with suitable barrier properties	Ayquipa-Cuellar et al. (2020)

contained residual amounts of polyphenols, which can interact with reactive groups of the same mucilage. Consequently, they observed a reduction of film free volume and led to higher water barrier properties. Plasticizer incorporation into the matrix polymer decreases the intramolecular interactions between polymer chains in favor of hydrogen interactions. Plasticizers with different molecular sizes increase polymer segments' mobility, which may enhance the diffusivity of water vapor through the film matrix. Glycerol films were more flexible and with a low crystal transition, while sorbitol films were stronger and more significant barriers to WVP.

Cladode mucilage exhibited suitable film-forming properties and may have potential applications in food packaging. *Opuntia* cladodes mucilage is safe, biodegradable and available at low cost. Guadarrama-Lezama et al. (2018) combined mucilage with citric pectin to make composite edible films. Mucilage improves film mechanical properties like elongation, shear force, tension, and elasticity. In film formation, mucilage reducing carbohydrates crystallizes as processing temperature increases, generating films with compact structures. Therefore, mucilage presents various endothermic transitions, which suggests the interaction between pectin and carbohydrates through hydrogen bridges with the OH groups of both molecules increases melting temperature. This can limit the water transport through the film, improving the ability to retain water. Mucilage molecules reinforce a three-dimensional network of the microstructure formed by the citric pectin with the plasticizer. Microstructure analysis revealed a homogeneous network, with reducing pores and microphases. When these polymers are mixed, they exhibit two types of interactions (attraction or repulsion). It depends on the solvent, charge groups distribution, and hydrophilic or hydrophobic groups. In this study, aggregates formation could be attributed to electrostatic interactions between pectin and mucilage.

Further, Mujtaba et al. (2019) established that composite films' thermal stability is important for its use and processability wherever melt mixing is considered. The tensile and loss modules showed a heterogeneous phase and partial separation when mucilage is mixed with the plasticizer and water. Mucilage, like other polysaccharides, is a hard and brittle polymer and should be plasticized with glycerol or another polyol to increase the flexibility and workability in edible film form.

Cenobio-Galindo et al. (2019) developed starch films added with microencapsulated extracts from the *Opuntia oligacantha* fruit called xoconostle. Its consumption has a significant content of phenolics. Extracts microencapsulation and edible films provide protection and preservation of antioxidant activity and phenols concentration. Gheribi et al. (2019a) mixed different amounts of mucilage with polyvinyl alcohol (PVA) to produce edible films. Combinations provided the ability to prevent or reduce the passage of UV-Vis light. The mixture reinforces various mechanical properties such as shear stress and elongation. Such mechanical resistance improvement may be related to the intramolecular interactions occurring between mucilage and PVA, leading to a stronger and more cohesive structure. These interactions were defined as hydrogen bonds occurring between PVA hydroxyl and mucilage carboxyl groups. These composites films could represent a promising application for food packaging materials. Sandoval et al. (2019) made edible mucilage films from different varieties of *O. ficus-indica*. Capena variety was the one that presented the



best results in transparency, resistance, and light transmission rate parameters. Mucilage solubility is essential because if applied as a coating, it will be consumed with the product. Low solubility indicates a greater cohesion in the polymer matrix, mainly because of the formation of many hydrogen bonds between the polymers chain and water. Mucilage contains 35–40% arabinose, 20–25% galactose and xylose, and 7–8% rhamnose and galacturonic acid. Besides, water-soluble polysaccharides are long-chain polymers that dissolve or disperse in water, conferring a viscous effect.

*Opuntia* by-products constitute a promising source of film polymers. Aparicio-Fernández et al. (2018) developed composite edible films, mixing carboxymethyl cellulose (CMC) with red prickly pear peel powder (*O. ficus-indica*) cv. San Martín considered a vegetable waste. These by-products maintain significant concentrations of bioactive compounds. Flexible films with a high content of the antioxidant activity and reducing power were formed. A potential functional polymer with valuable antioxidant compounds can be incorporating into biodegradable food packaging materials. Further, Gheribi et al. (2019b) investigated the film-forming ability from prickly pear peel. Films micrographs exhibited compact, homogeneous, and smooth surfaces, with high water-vapor barrier properties. They showed higher moisture content and lower solubility than films based on mucilage. Furthermore, it is, therefore, by the presence of phenolic and waxy compounds in peel mucilage. The structure rich in hydroxyl groups and a pseudoplastic behavior of the film-forming solution promote interactions within the film matrix and lead to a homogeneous film structure.

Ayquipa-Cuellar et al. (2020) produced edible films from prickly pear peel by-products. Obtained films presented suitable barrier properties due to low WVP. FTIR and SEM evidenced the molecular interaction of the components through hydrogen bonds. Most of peel carbohydrates are neutral, while some gums are negatively charged because of considerable numbers of hydroxyl groups or other hydrophilic moieties in the neutral carbohydrate structure. Hydrogen bonds play the principal role in film formation and characteristics. Research also has been developed in coated food. In this context, Del-Valle et al. (2005) reported that *O. ficus-indica* mucilage was used as an edible coating of strawberries. The mucilage coating improved the firmness, sensory properties, and extended the shelf-life of strawberries. Mucilage has also been used as an edible coating in kiwifruit slices (Allegra et al., 2016). Both authors reported that the coating with *O. ficus-indica* mucilage maintained firmness, visual quality, and reduced weight loss. Zambrano-Zaragoza et al. (2014) coated red delicious apple slices with a mixture of tocopherols with nopal mucilage in the form of nanoemulsions. With this type of functional coating, they evaluated the effect of enzymes that cause enzymatic browning, observing a decrease in the slices respiratory rate, achieving a significant reduction in oxidation degree and firmness maintenance. They conclude that mucilage helps to control browning index and, therefore, with significant potential of functional nanocoatings preparations.

Allegra et al. (2017) coated breba figs or ‘dottato’ (*Ficus clarica* L.) with a mucilage solution, showing an improvement in coated fruit overall quality. Coating

maintains firmness, carotenoid concentration, and low development of *Enterobacteriaceae* during storage. The interaction with coating and low temperatures reduces water transpiration. Fruits kept for 10 days in acceptable quality conditions. Treviño-Garza et al. (2017) used the layer-by-layer technique to coat pineapple cubes by mixing chitosan, pullulan, linseed, mucilage, and *Aloe vera*. They were suitable to extend the product shelf life. Multilayers coating offered microbiological stability. Mucilage can perform water-binding capacity, which allows it to have less water loss due to steam and weight, improving the fruit's firmness. Besides, these coatings form a physical and mechanical barrier that delays respiratory metabolism and decreases water loss by dehydration.

Regarding enzymatic activities which are related to fruit softening. Little change in color and off-flavor development were detected. Finally, Wu (2019) developed *Opuntia dillenii* edible coatings onto potato slices. This type of semi-permeable film exhibit antioxidant and antimicrobial activity. It could extend the product shelf life. These coatings inhibited potatoes browning, respiration reduction, and weight loss during storage. The main components of *O. dillenii* polysaccharides are arabinose, xylose, fructose, glucose, galacturonic acid, and rhamnose.

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## Chapter 40

# Microencapsulation Technology: An Alternative Preservation Method for *Opuntia* spp. Derived Products and Their Bioactive Compounds



Zeynep Aksoylu Özbek, Pelin Günç Ergönül, and Bilge Taşkın

**Abstract** Microencapsulation is considered a convenient preservation method for sensitive food components. *Opuntia* spp. cladodes contain substantial amounts of phytochemicals, mainly flavonoids, betalains, and carotenoids. Due to legal restrictions on the use of synthetic colorants, food technologists are seeking alternative natural sources to be served as a food colorant. The presence of pigments, namely betalains and carotenoids, which are also well-known antioxidants, make *Opuntia* spp. an excellent food colorant agent. However, various factors, including pH, moisture, light, oxygen, temperature, and enzymatic activities, promote betalain and carotenoids' degradation. Therefore, studies have been focused on the application of different microencapsulation techniques to *Opuntia* spp. derived products such as fruit juice, pulp, or phytochemicals-rich extracts. Spray drying is the most common method for encapsulation of *Opuntia* spp. derived products. Moreover, freeze-drying and ionic gelation are the other encapsulation techniques used in *Opuntia* spp. products. The feasibility of maltodextrin (MD), modified starch, gum arabic, inulin, glucose syrup, gelatin, and cladode mucilage as wall material for encapsulation *Opuntia* spp. derived products have been evaluated up to now. Besides the preservation of bioactive compounds, microencapsulation provides the conversion of liquid state food into powdered form, facilitating its storage and extending its areas of use. The researchers agree that microencapsulation is a promising preservation method for *Opuntia* spp. bioactives, particularly betalains. Furthermore, microencapsulated *Opuntia* spp. derived products may be incorporated into various food product formulations such as extruded snacks, soft drinks, edible films, gummy candies, and yoghurt to obtain an attractive color and enhance the antioxidant capacity of food items. This review will discuss the microencapsulation studies related to *Opuntia* spp. derived products, including fruit juice, pulp, and extracts.

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**Keywords** Inulin · Maltodextrin · Microencapsulation · Micro particle · Food technology

## Abbreviations

AHA	American Heart Association
BE	Betanin equivalent
BSA	Bovine serum albumin
CE	Catechin equivalent
DE	Dextrose equivalent
DP	Degree of polymerization
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
GAE	Gallic acid equivalent
IE	Indicaxanthin equivalent
LDL	Low-density lipoprotein
MD	Maltodextrin
NHMRC	Australian National Health and Medical Research Council
QE	Quercetin equivalent
RH	Relative humidity
SEM	Scanning electron microscopy
SPI	Soy protein isolate
TE	Trolox equivalent
Tgase	Transglutaminase
WHO	World Health Organization

## 1 Introduction

Cladodes, pads, nopales, or pencas are the edible stems of plants that belong to *the Opuntia* genus (Astello-García et al., 2012). The cladodes of different *Opuntia* spp. cultivars including *Opuntia streptacantha*, *Opuntia hyptiacantha*, *Opuntia megacantha*, *Opuntia albicarpa*, and *Opuntia ficus-indica* has been reported to be rich sources of protein (9.34–12.6%), crude fiber (5.35–7.88%), minerals (mainly potassium and calcium), phenolics and flavonoids (Astello-García et al., 2015). The fruits of *Opuntia* spp. plants are known as prickly pear or cactus pear. They contain substantial amounts of ascorbic acid, phenolics, flavonoids, flavonols, betaxanthins, and betacyanins (Reis et al., 2017; Betancourt et al., 2017; García-Cayuela et al., 2019). These bioactive compounds also contribute to the unique antioxidant capacity of *Opuntia* spp. fruits (Osorio-Esquivel et al., 2011; Yahia & Mondragon-Jacobo, 2011; Moussa-Ayoub et al., 2014).

One of the most attractive aspects of *Opuntia* spp. fruits is to be used as a source of natural colorants due to their high betalains contents (Castellanos-Santiago & Yahia, 2008). Betalains are water-soluble pigments divided into yellow/orange betaxanthins and red/purple betacyanins (Strack et al., 2003). Betalains have anti-oxidant, anti-fibrotic, anti-inflammatory, antimicrobial, antiviral, and apoptotic effects. Furthermore, they are beneficial for reducing blood pressure, cholesterol, and low-density lipoprotein (LDL) as well as the health of the cardiovascular, nervous, and gastrointestinal systems (Delgado-Vargas et al., 2000; Rahimi et al., 2019). Indicaxanthin, vulgaxanthin I, vulgaxanthin IV and muscaaurin VII (betaxanthins), neobetanin, betanin, isobetanin, gomphrenin I, and betanidin (betacyanins) have been identified in *Opuntia* spp. fruits (Strack et al., 1987; Fernández-López & Almela, 2001; Stintzing et al., 2002, 2005). However, the stability of betalain pigments depends on various factors, as illustrated in Fig. 40.1.

Different preservation techniques such as the addition of chelating agents [ethylenediaminetetraacetic acid (EDTA), citric acid] or antioxidants (ascorbic acid), encapsulation, and a combination of high-pressure processing and anionic

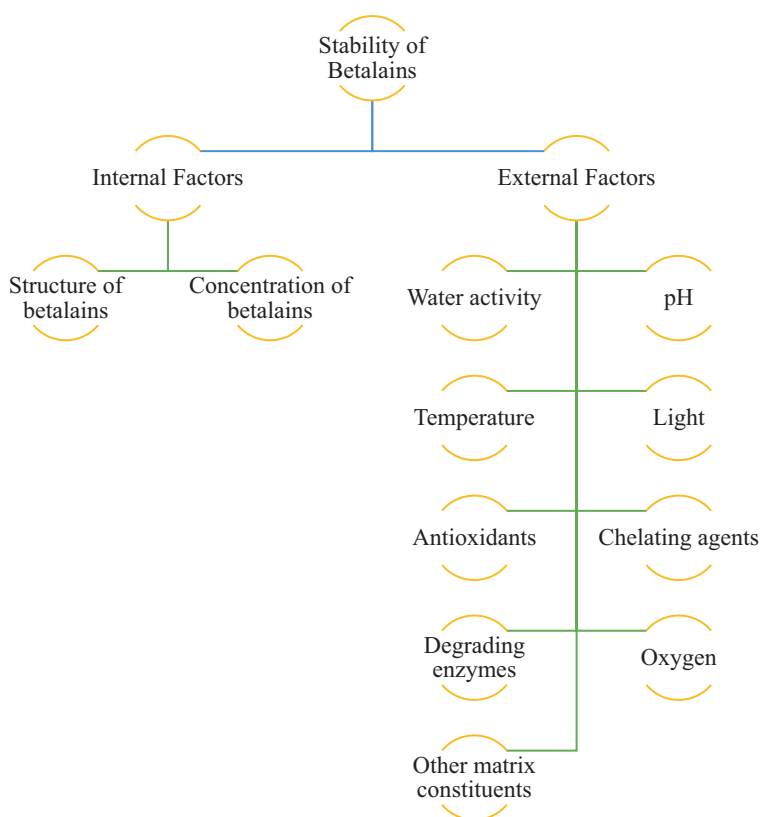


Fig. 40.1 Factors affecting the stability of betalains (Herbach et al., 2006; Azeredo, 2009)

polysaccharide complexation have been applied to improve the stability of betalain-rich fruits and vegetables (Gandía-Herrero et al., 2010, 2013; Pitalua et al., 2010; Skopińska et al., 2012, 2014, 2015a, b; Szot et al., 2013; Ravichandran et al., 2014; Rodriguez et al., 2015; Otálora et al., 2015, 2016; Kaimainen et al., 2015; Tumbas Šaponjac et al., 2016; Giridhar, 2017; Juan Antonio Rodríguez-Sánchez et al., 2017; Selig et al., 2018; Pagano et al., 2018; Zuanon et al., 2019; Robert et al., 2020; Utpott et al., 2020). Among these techniques, microencapsulation is one of the most promising and widely used methods to prevent betalain degradation. In this chapter, microencapsulation studies using various parts or extracts of *Opuntia spp.* plants as core material are discussed.

## 2 Microencapsulation

Encapsulation is a process that reduces the sensitivity of solid, liquid, or gaseous materials against detrimental extrinsic factors by entrapping core materials in suitable matrices called wall materials (Desai et al., 2005; Zuidam & Shimoni, 2010). Different terms such as microencapsulation (with a diameter of 3–800µm) or nanoencapsulation (with a diameter of 10–1000 nm) are used depending on the size of particles obtained through the encapsulation technique (Kwak, 2014). In the food industry, encapsulation provides

- Protection of bioactive compounds by decreasing their interaction with the surrounding atmosphere,
- Controlled release of bioactive compounds or microorganisms like probiotics,
- Masking undesirable flavor,
- Better handling and storage properties of core materials,
- Modification of core materials' properties such as water/oil solubility and color (Zuidam & Shimoni, 2010; Nedović et al., 2011, 2013).

Encapsulation techniques are mainly divided into three groups:

- (a) Physical methods (spray drying, freeze-drying, spray freeze drying, fluidized bed coating, extrusion, co-crystallization, centrifuge extrusion)
- (b) Chemical methods (molecular inclusion, polymerization)
- (c) Physicochemical methods (coacervation, liposomes) (Shahidi & Han, 1993; Bansode et al., 2010; Sri et al., 2012; Fang & Bhandari, 2012; da Silva et al., 2014).

*Opuntia spp.* derived products are generally encapsulated in order to reduce the degradation rate of their bioactive compounds.

## 2.1 Microencapsulation of *Opuntia* spp. Pulp

The main part of *Opuntia* spp. fruits is the edible pulp (flesh), and 15% of total pulp weight is constituted by the seeds (Barba et al., 2020). The pulp ratio (%) of *Opuntia* spp. fruits are summarized in Table 40.1.

Various *Opuntia* (*Opuntia ficus indica*, *Opuntia matudae*, *Opuntia dillenii*, and *Opuntia joconostle*) fruits pulps are rich in carbohydrates (glucose and fructose), fiber (pectin, hemicellulose, cellulose), minerals (calcium, potassium, magnesium, and sodium), vitamin C and bioactive compounds including phenolics, carotenoids (all-E-lutein, (all-E)- $\beta$ -carotene, (all-E)-violaxanthin, (all-E)-neoxanthin, (all-E)-antheraxanthin, (all-E)-zeaxanthin, lutein-5,6-epoxide) and betalains (betacyanins such as betanin and isobetanin and betaxanthins such as indicaxanthin, aminobutyric acid-betaxanthin, muscaarin, isoleucine betaxanthin, vulgaxanthin) (El Kossori et al., 1998; Medina et al., 2007; Guzmán-Maldonado et al., 2010; Morales et al., 2012; Cejudo-Bastante et al., 2014; Cano et al., 2017).

Different colors of *Opuntia* spp. fruit pulps are due to the presence of natural pigments like carotenoids and betalains. Nevertheless, the sensitivity of these compounds against environmental factors requires additional processes. Attempts have been made for encapsulation of *Opuntia* spp. fruit pulps by different techniques. In a study, both *Opuntia ficus indica* pulp and its ethanolic (EtOH/water, 1:1) extract were encapsulated with different amounts of maltodextrin (MD) (DE 10) or inulin (DP > 23) (6–30% and 3–15%, respectively) through spray drying. The optimum microencapsulation parameters were reported as follows: the core-to-wall material ratio of 3:1 and inlet temperature of 140 °C for MD containing pulp (MD-P) and extracts (MD-E); the core-to-wall material ratio of 3:1 and 5:1 for inulin containing pulp (IN-P) and extracts (IN-E), respectively and inlet temperature of 120 °C for pulp and extracts including inulin. Independently of wall material type used, microencapsulation of *Opuntia ficus indica* pulps resulted in remarkable recovery of betacyanins (100%), indicaxanthins (100%), and polyphenols (112–119%), whereas microencapsulation of pulp extracts decreased the retention of betacyanins (62–81%) and indicaxanthins (67–86%). During storage of microcapsules produced under optimized conditions at 60 °C for 44 days, total betacyanins contents

**Table 40.1** Pulp ratio of different *Opuntia* spp. fruits

<i>Opuntia</i> spp.	Growth location	% Pulp	Reference
<i>Opuntia crassa</i> Mexico	USA	46.40	Felker et al. (2002)
<i>Opuntia crassa</i> Mexico	Argentina	50.40	
<i>Opuntia streptacantha</i>	USA	52.30	
<i>Opuntia streptacantha</i>	Argentina	59.10	
<i>Opuntia ficus indica</i>	USA	39.20–64.20	
<i>Opuntia ficus indica</i>	Argentina	43.20–68.50	Felker et al. (2005)
<i>Opuntia ficus indica</i>	Argentina	40.30–47.20	
<i>Opuntia dillenii</i>	Spain	58	
<i>Opuntia ficus indica</i>	South Africa	44.39–62.84	de Wit et al. (2010)

decreased by 37%, 41%, 46%, and 40% in MD-P, IN-P, MD-E, and IN-E, respectively. Accordingly, total indicaxanthins contents were reduced by 27%, 37.50%, 50%, and 20% in MD-P, IN-P, MD-E, and IN-E. Apart from these, encapsulation with MD caused a reduction in total phenolics amounts of microcapsules (11.8–22%), while inulin led to higher total phenolics contents at the end of storage (from 2.26 to 3.00 mg GAE/g in pulp-containing microcapsules and from 2.41 to 3.81 mg GAE/g in extract-containing microcapsules). The authors concluded that pulp-based microcapsules provided better retention of bioactive compounds as other pulp components (particularly mucilage) act as a key determinant in the microencapsulation process (Saéñz et al., 2009). However, this finding is in contrast to the findings of Vergara et al. (2014), who declared that compared to ultrafiltered *Opuntia ficus indica* pulp extract microparticles, at 60 °C higher betanin degradation rate in spray-dried *Opuntia ficus indica* pulp microparticles containing modified corn starch as wall material may be related to the mucilage and/or high sugar content of the pulp. These two components may contribute to the increase of available water content that induces betanin degradation by specific hydrolysis reactions (Herbach et al., 2006; Vergara et al., 2014).

Novel wall materials have been examined as an alternative to common ones like MD, gum arabic, and whey protein. For this purpose, oca starch's suitability extracted from *Oxalis tuberosa* tubers was investigated in spray or freeze-dried microcapsules, including extract of *Opuntia ficus indica* pulp as core material. In this study, oca starch was used alone or in combination with MD (DE 10). Spray-dried microcapsules containing oca starch: MD in a ratio of 70:30 preserved the total phenolics (retention of 63.5%), antioxidant activity, and betacyanins (retention of 86.5%) better during 105-days storage under dark at room temperature. The researchers explained this finding by preventing oxygen penetration through capsules due to the addition of high molecular weight polymers like oca starch. Besides, freeze-drying yielded particles with irregular surfaces and greater sizes, enabling the degradation of bioactive compounds, particularly betacyanins. Therefore, a wall material combination consisting of 70% of oca starch and 30% of MD and spray drying as a microencapsulation technique was recommended to fabricate *Opuntia ficus indica* pulp extract powders with improved stability and higher bioactives retention (Morales et al., 2020).

Using a combination of wall materials is a common approach in microencapsulation technology. The feasibility of the blends of soy protein isolate (SPI), MD (DE 10), and inulin (DP > 23) for encapsulation of *Opuntia ficus indica* pulp by spray drying was evaluated by Robert et al. (2015). The results of the study demonstrated that, in comparison to SPI alone, the coupling of this protein with carbohydrates (MD or inulin) improved encapsulation efficiencies of betacyanins (from 99.6% to 99.9%), betaxanthins (from 98.1% to 99.5%), and polyphenols (from 79.7% to 86.5%) as well as storage stability of these bioactive owing to the film-forming properties of carbohydrates. However, the blend including SPI/MD provided higher betacyanin and betaxanthin stability at 60 °C for 56 days than SPI/inulin. Even though both MD and inulin are polysaccharides, the different stabilities of microcapsules may be linked with these polymers' structural differences (Robert et al.,

2015). Similarly, another research findings showed that the incorporation of *Opuntia ficus indica*'s cladode mucilage to MD-based (DE 20) wall material enhanced the encapsulation yield of *Opuntia ficus indica* pulp extract by preventing the product adhesion into the drying chamber of the spray dryer. Additionally, the presence of cladode mucilage ensured the formation of more spherical and uniform particles with a smoother surface due to its high molecular weight. On the other side, microcapsules containing MD alone as wall material exhibited better betanin retention thanks to their denser wall structure, which provides a better oxygen barrier (Otálora et al., 2015). A recent study aimed to produce microparticles from *Opuntia ficus indica* pulp by using MD, *Opuntia ficus indica* cladode mucilage, and MD/cladode mucilage through spray drying technique. The highest indicaxanthin retention (100%) and yield (51.2%) were determined in the powders containing MD, while the best betaxanthins protection (19.8 mg indicaxanthin equivalent/100 g) was provided by cladode mucilage. Throughout 49-days storage at 60 °C under dark conditions, 90%, 40%, and 60% of indicaxanthins retained in the microparticles containing MD, cladode mucilage, and MD/cladode mucilage as wall material, respectively. Furthermore, MD and MD/cladode mucilage incorporated microparticles successfully served as natural colorants in yoghurt with indicaxanthin retention rates of 83.4% and 82% at 4 °C for 28 days, respectively. Based on the study's findings, the authors concluded that MD/cladode mucilage mixture is a suitable wall material for encapsulation of yellow/orange colored *Opuntia ficus indica* pulp (Carmona et al., 2021).

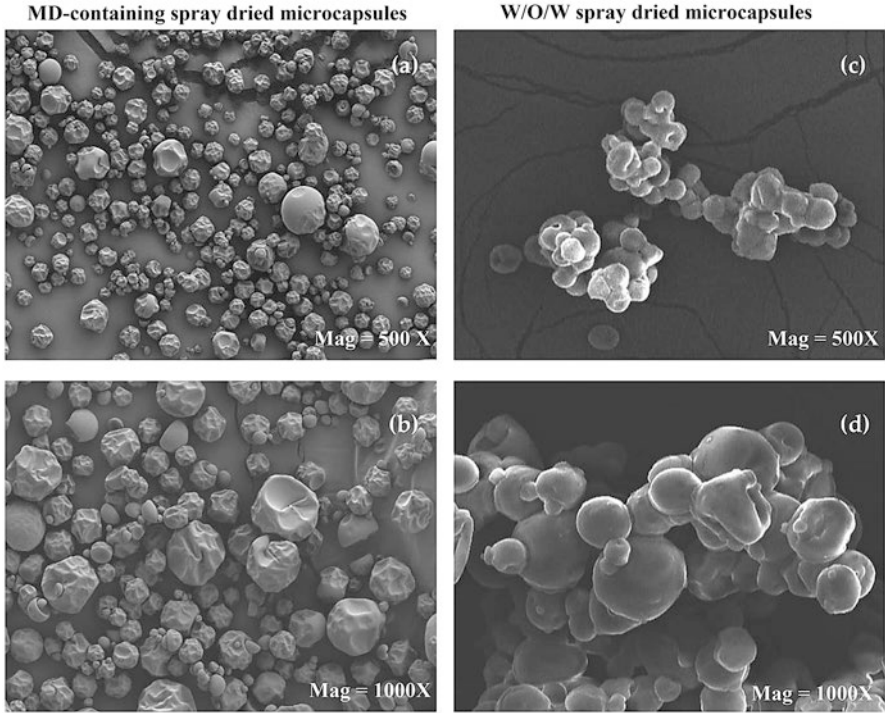
Another typical encapsulation method is ionic gelation. Few attempts have been made for the fabrication of *Opuntia* spp. powders by using this technique. Firstly, calcium alginate and calcium alginate/bovine serum albumin (BSA) beads were introduced for *Opuntia ficus indica* pulp extract microencapsulation. The incorporation of BSA did not lead to any change in size and moisture contents of the beads. The researchers reported that betalain stability at 25 °C depended on the relative humidity (RH) of the environment and bead matrix composition. Irrespective of bead type, betalain retention decreased as the moisture of the storage environment increased. The maximum betalain retention (48.8%) was obtained at 34.6% RH for calcium alginate beads, whereas storage at 57.6% RH provided the best betalain retention (39.9%) in calcium alginate/BSA beads.

Nonetheless, both bead types were found to be suitable to produce natural colorant additive from *Opuntia ficus indica* pulp (Otálora et al., 2016). Afterward, the same research group carried out a different study to encapsulate *Opuntia megacantha* pulp extract by ionic gelation and spray drying (Otálora et al., 2018). For spray drying, a combination of MD (DE 20) and cladode mucilage was used as wall material, while sodium alginate was the matrix for hydrogel beads. The spray-dried microcapsules had non-smooth spherical or spheroidal surfaces as the formation of regular spheres was avoided by high molecular weight cladode mucilage. In contrast, ionic gelation followed by air-drying at 30 °C generated irregular microparticles with rough and cracked surfaces because of bead wall collapsing during water removal. At the end of 30-days storage (at 90% or 57% RH), spray-dried microcapsules retained betaxanthins to a greater extent than that produced through ionic

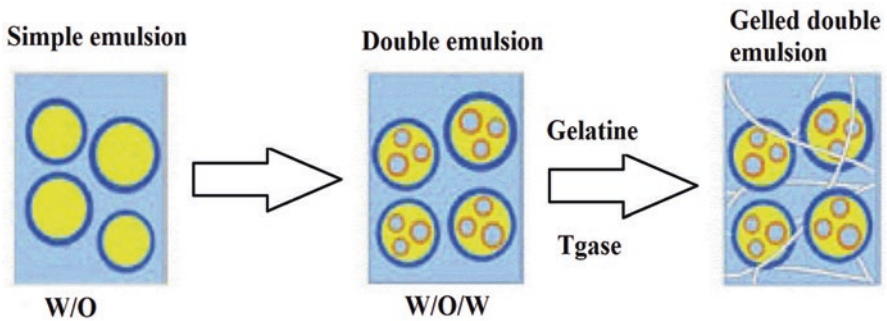
gelation. The researchers attributed this to the highly hydrophilic character of sodium alginate, which triggers capsules' deterioration. Besides, orange/yellow-colored betaxanthins were more stable to degradation reactions during storage than red/purple-colored betacyanins (Otálora et al., 2018).

Double emulsions are defined as complex multi-phase dispersions in which the droplets of one dispersed liquid are further dispersed in another liquid (Garti & Benichou, 2004; Kim et al., 2018). To produce low-fat foods and improve the delivery of some nutrients, bioactives, and flavors, double emulsions have been widely used for a long time (Muschiolik & Dickinson, 2017). These emulsion systems are mainly grouped into two categories: water-in-oil-in-water (w/o/w) and oil-in-water-in-oil (o/w/o) emulsions (Garti & Bisperink, 1998). The number of studies using double emulsions to encapsulate the extracts of *Opuntia* spp. fruits are scarce. One of them aimed to compare the attributes of spray-dried *Opuntia ficus indica* fruit extract microcapsules obtained through either w/o/w emulsion system or MD-containing solution (conventional technique). The authors reported that phenolic compounds' encapsulation efficiency values were in the range of 95–97% and 67–70% for conventional and double emulsion systems, respectively (Toledo-Madrid et al., 2018). As it is seen, MD-containing solution preserved the phenolics better during the spray drying process. This finding may be correlated with the environmental stresses induced by several food processes, including drying, freezing, and chilling, which may easily breakdown the physical stability of double emulsions (McClements, 2010). Accordingly, greater phenolics retention in spray-dried microcapsules was achieved using only MD (91–107%) than w/o/w emulsion (74–82%). However, double emulsions produced microparticles with superior antioxidant capacity (100%) compared to MD-containing particles (>94%). In summary, microcapsules obtained through a double emulsion system had lower phenolics but higher antioxidant capacity (Toledo-Madrid et al., 2018). Indeed, since the antioxidant activity is not just a function of phenolic compounds, this observation is not surprising. Furthermore, each phenolic compound's contribution to the overall antioxidant capacity of any food is not equal (Zhao et al., 2019). Other antioxidative compounds rather than phenolics, particularly betalains, may also contribute to the microcapsules' total antioxidant capacity containing *Opuntia ficus indica* fruit pulp extract (Belhadj Slimen et al., 2017). In terms of morphological attributes (Fig. 40.2), spray-dried particles containing MD had dented and spherical surfaces, whereas double emulsions produced considerably agglomerated particles with smooth and spherical surfaces (Toledo-Madrid et al., 2018).

The most frequently encountered problem in double emulsions prepared for food-intended uses is their very low thermodynamic stability (Garti, 1997; Dickinson, 2011). The ability of various proteins to form a gel structure makes them excellent agents to improve double emulsions' stability. Gelation may be induced by heat treatment or cold gelation process. Cold gelation consists of two stages: (a) protein unfolding by pre-heating and (b) gel formation by addition of acidifier, divalent salts, or enzymes (mainly transglutaminase (Tgase)) (Mantovani et al., 2016). Nevertheless, heating-induced gels' stabilization of emulsions is not favorable for encapsulation or delivery of heat-sensitive compounds, including prebiotics,



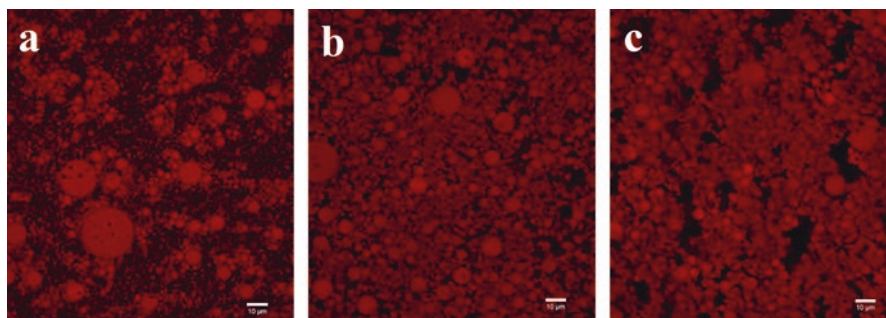
**Fig. 40.2** Scanning electron microscopy (SEM) images of spray-dried *Opuntia ficus indica* fruit extract microcapsules obtained from MD-containing (conventional) wall solution and w/o/w (double) emulsions. (Adapted from Toledo-Madrid et al. (2018))



**Fig. 40.3** Stabilization of  $W_1/O/W_2$  double emulsion by cold-set gelation. (Adapted from Flaiz et al. (2016) with permission)

vitamins, flavors, and various nutraceuticals (Liang et al., 2010; Ruffin et al., 2014). Therefore, cold-set gelation is a promising alternative as it enables the stabilization of heat-labile compounds by gel formation without any thermal treatment (Brito-Oliveira et al., 2017). Gelled double emulsion formation is illustrated in Fig. 40.3.





**Fig. 40.4** Microstructure of (a) conventional double emulsion (DE-CP), (b) gelled double emulsion containing bovine gelatin (DE-CP-G), and (c) gelled double emulsion containing bovine gelatin and microbial transglutaminase (DE-CP-GT). (Adapted from Robert et al. (2020))

In a very recent research study, ethanolic extract of *Opuntia ficus indica* pulp was encapsulated in cold-gelled double emulsions containing bovine gelatin (DE-CP-G) or a combination of bovine gelatin and microbial Tgase enzyme (DE-CP-GT). As shown in Fig. 40.4, all double emulsions had a typical multi-compartmentalized structure, but the addition of gelatin (DE-CP-G) and Tgase (DE-CP-GT) contributed to more closely packing of oil globules. The findings of this study showed that the gelatin/Tgase mixture provided a thermostable gel. In conventional double emulsion (DE-CP), betalain retention decreased from 100% to 48.6% at the end of 49-days storage at 4 °C. Under the same conditions, DE-CP-G maintained 59% of the initial betalain content, whereas only 6.80% of initial betalains was detected in DE-CP-GT on the 28th day of storage. This result supported that Tgase enzyme activity led to unfavorable pH conditions (>7) that trigger betanin degradation. Betanin degradation was also confirmed by shifting emulsions color from red/purple to yellow fades throughout storage since betanin degradation products betalamic acid, and a yellow color characterizes neobetainidin 5-O- $\beta$ -glucoside, and 17-decarboxy betanin has orange/red color. Hence, the application of gelled double emulsions consisting of only gelatin was recommended for improved betanin stability (Robert et al., 2020).

## 2.2 Microencapsulation of *Opuntia* spp. Peel

The peels of *Opuntia ficus indica* fruits (prickly pear) contain macronutrients such as fibers (mainly cellulose and hemicellulose), carbohydrates (glucose, fructose, and saccharose), minerals (potassium, calcium, magnesium, and manganese), and micronutrients like phenolic compounds (piscidic acid, pimelic acid, eucomic acid) and pigments (El Kossori et al., 1998; Aruwa et al., 2019). Furthermore, different betalains and carotenoid patterns were detected in the peels depending on prickly pears' color. For instance, only the peel of red-colored cultivar includes betacyanins

(betanin, isobetanin, betalamic acid, 2,15,17-tridecarboxy-neobetanin, 2-decarboxy-neobetanin, 2-decarboxy-betanin) and betaxanthins (proline-betaxanthin, histidine-betaxanthin). In contrast, those of yellow/orange-colored (isoleucine-betaxanthin, dopamine-betaxanthin, dopa-betaxanthin, serine-betaxanthin), while green-colored (dopa-betaxanthin, serine-betaxanthin, tyramine-betaxanthin) varieties include only betaxanthins. On the other hand, more carotenoid compounds were identified in the peel of green-colored *Opuntia ficus indica* fruits (neurosporene, physallen, violaxanthin,  $\alpha$ -carotene) than those of red-colored and yellow/orange-colored ones (Amaya-Cruz et al., 2019). Moreover, carotenoids contents of the peel (1257.74–1693.38 $\mu$ g/100 g) of *Opuntia ficus indica* fruit were reported to be higher than its pulp (255.93–379.45 $\mu$ g/100 g) and whole fruit (444.90–478.11 $\mu$ g/100 g) (Cano et al., 2017). Nevertheless, all these biologically active components are prone to degradation reactions. Therefore, many attempts have been made to maintain the stability of these compounds.

Spray drying is the most preferred microencapsulation technique in the food industry due to flexibility of process parameters, lower production costs, and availability of the equipments (Desai et al., 2005; Gharsallaoui et al., 2007). The use of MD (DE 10) or gum arabic in various ratios (10, 15, and 20% wt.) was investigated for the microencapsulation of *Opuntia ficus indica* peel extract spray drying. It was shown that MD protected phenolic compounds better against oxidation and therefore yielded microcapsules with higher antioxidant capacity than gum arabic. In contrast, gum arabic provided greater betacyanins content than MD due to its higher film-making capability and hydrophilic nature. In general, regardless of the type of wall material used, wall material addition at a ratio of 10% wt. contributed to higher retention of betacyanins, betaxanthins, and antioxidant capacity during 90-days storage in the presence or absence of light. Additionally, the authors determined the optimum spray drying conditions as follows: 170 °C and 80 °C for inlet and outlet temperatures and wall material (MD or gum arabic) at a ratio of 10% wt. (Toledo-Madrid et al., 2019).

Encapsulated bioactive compounds may be used as natural preservatives in various food products. For this purpose, Egyptian *Opuntia ficus indica* L. fruit peels were nano encapsulated in chitosan, sodium alginate, and a combination of chitosan/sodium alginate. Encapsulation with chitosan resulted in the maximum encapsulation efficiency (97.8%), while the lowest encapsulation efficiency (68.5%) was reported for sodium alginate-based nanoparticles. However, except for sodium alginate (5% loss), the encapsulation process caused an insignificant reduction in total phenolics content of ethanolic extract of *Opuntia ficus* peels. The authors incorporated both the unencapsulated (native form) and encapsulated (nanoparticles) ethanol extract of *Opuntia ficus indica* peels in guava juice as natural antioxidant sources. The study's findings showed that sodium alginate-based nanoparticles improved the sensory quality of guava juice, whereas the chitosan/sodium alginate combination reduced the sensory acceptability of the same food product (Mahmoud et al., 2018).

### 2.3 Microencapsulation of *Opuntia* spp. Juices

As in most fruits, *Opuntia* spp. cultivars' fruits have extremely high levels of water. For instance, water contents of *Opuntia ficus indica* fruit's pulp and skin were reported to be 94.40% (w/w) and 90.33% (w/w), respectively (Salim et al., 2009). Some physicochemical characteristics of fruit juices obtained from various *Opuntia* cultivars were summarized in Tables 40.2 and 40.3. Gallic acid, syringic acid, ellagic acid, catechin, epicatechin, procyanidin B1, procyanidin B2, quercetin, and isorhamnetin derivatives are the phenolic compounds identified in juices of *Opuntia ficus indica*, *Opuntia albicarpa*, *Opuntia megacantha*, *Opuntia streptacantha*, *Opuntia streptacantha* ssp. *aguirrana*, and *Opuntia robusta* (Mata et al., 2016; Zenteno-Ramírez et al., 2018). Additionally, *Opuntia ficus indica* fruit juices are rich in betalains [indicaxanthin, betanin, isobetanin, tyrosine betaxanthin, leucine-betaxanthin (vulgaxanthin IV), tryptophan-betaxanthin] (Mata et al., 2016). Studies have shown that juices of *Opuntia* spp. fruits have an inhibitory effect on the colon, prostate, and hepatic cancer cells owing to their superior bioactive composition (Chavez-Santoscoy et al., 2009).

Spray dryer is the most common equipment used in encapsulation studies. Nevertheless, presence of low molecular weight sugars (fructose, glucose, sucrose) and organic acids (malic acid, citric acid, tartaric acid) that dominates fruit juices may cause stickiness problem during the spray drying process (Bhandari et al., 1997; Bhandari & Adhikari, 2009). The addition of high molecular weight substances, which are generally used for microencapsulation, is the simplest approach

**Table 40.2** pH, acidity and Brix values of fruit juices of various *Opuntia* spp. plants

Cultivar	pH	Acidity (citric acid %)	Brix	Reference
<i>Opuntia ficus indica</i>	3.30–6.48	0.02–0.88	6.20–15.4	Gurrieri et al. (2000), Dehbi et al. (2014) and El Kharrassi et al. (2016)
<i>Opuntia megacantha</i>	3.80–5.73	0.14–0.62	1.63–14.2	Jiménez-Alvarado et al. (2015) and El Kharrassi et al. (2016, 2020)
<i>Opuntia dillenii</i>	3.67	–	–	Moussa-Ayoub et al. (2016)
<i>Opuntia aequatorialis</i>	5.73	–	16.2	El Kharrassi et al. (2020)
<i>Opuntia leucotricha</i>	4.27–5.77	0.13–0.27	8.42–12.5	Chavez-Santoscoy et al. (2009) and El Kharrassi et al. (2020)
<i>Opuntia joconostle</i>	3.01–3.05	–	4.19–5.01	Castro-Muñoz et al. (2018) and Gómez-Covarrubias et al. (2020)
<i>Opuntia robusta</i>	4.85–5.33	0.05–0.12	8.08–14.7	Chavez-Santoscoy et al. (2009)
<i>Opuntia streptacantha</i>	5.10–5.54	0.06–0.18	13.3–15.5	Rodríguez-Hernández et al. (2005) and Chavez-Santoscoy et al. (2009)
<i>Opuntia violaceae</i>	5.44	0.03	12.93	Chavez-Santoscoy et al. (2009)
<i>Opuntia rastrera</i>	4.94	0.22	9.18	Chavez-Santoscoy et al. (2009)

**Table 40.3** Bioactives contents of fruit juices extracted from different *Opuntia* spp. cultivars

	<i>Opuntia ficus indica</i>	<i>Opuntia megacantha</i>	<i>Opuntia dilleanii</i>	<i>Opuntia leucotricha</i>	<i>Opuntia joconostle</i>	<i>Opuntia robusta</i>	<i>Opuntia streptacantha</i>	Reference
Vitamin C	2.40–781.10 mg/L	14.20–24.60 mg/L	558–595 mg/L	5.50 mg/L	–	–	236.50 mg/L	Rodríguez-Hernández et al. (2005), Moussa-Ayoub et al. (2016, 2017), El Kharrassi et al. (2016) and Karabagias et al. (2019)
∑Phenolics	0.75–14.26 mg GAE/mL	165.60–176.60 mg GAE/mL	6.40 mg GAE/mL	–	0.03–9.15 mg GAE/mL	0.02–0.11 mg GAE/g	0.20 mg GAE/g	Galati et al. (2003), Chavez-Santoscoy et al. (2009), Dengiz and Zengin (2016), Moussa-Ayoub et al. (2016), Castro-Muñoz et al. (2018), Pascoe-Ortiz et al. (2019) and Gómez-Covarrubias et al. (2020)
∑Flavonoids	266–428 µg CE/mL	–	–	95.80–238 µg QE/g	169 µg QE/mL	193–338 µg QE/g	321.40 µg QE/g	Chavez-Santoscoy et al. (2009), Gouws et al. (2019) and Gómez-Covarrubias et al. (2020)
∑Betalains	0.27–1.89 mg/100 mL	642–1821 mg/100 g	–	–	1.99 mg/100 g	–	–	Jiménez-Alvarado et al. (2015), Castro-Muñoz et al. (2018), Gouws et al. (2019) and Hadj Sadok et al. (2019)

(continued)

Table 40.3 (continued)

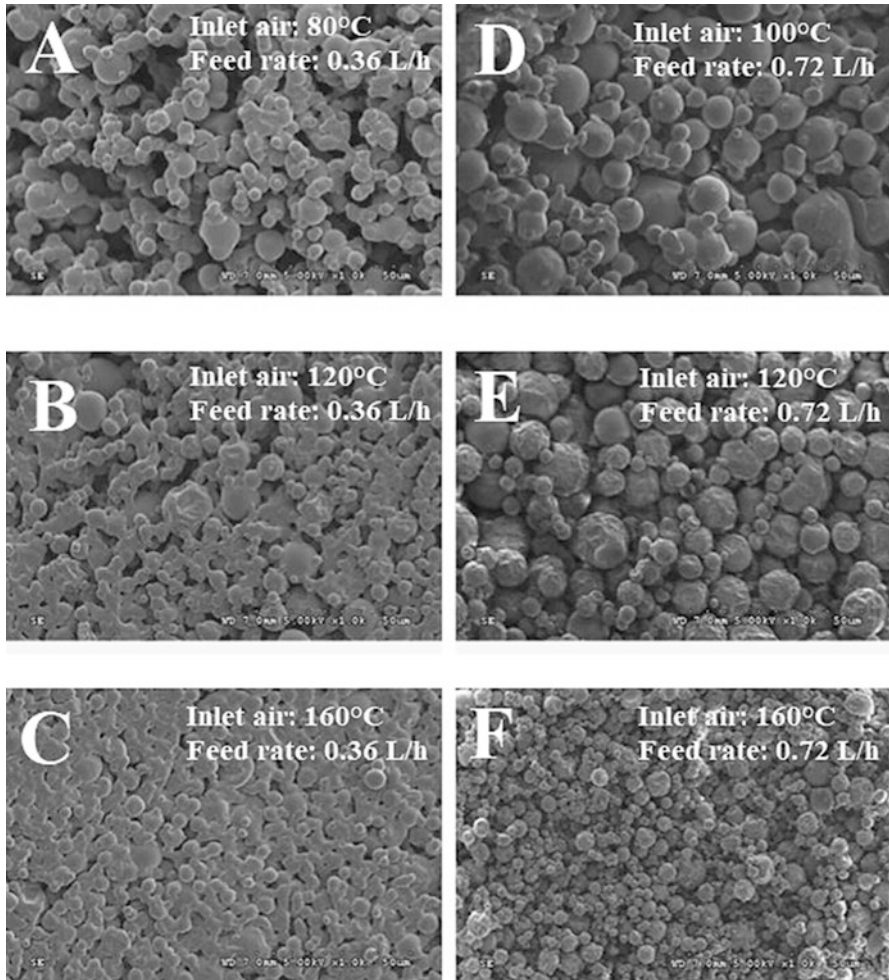
	<i>Opuntia ficus indica</i>	<i>Opuntia megacantha</i>	<i>Opuntia dillenii</i>	<i>Opuntia leucotricha</i>	<i>Opuntia joconostle</i>	<i>Opuntia robusta</i>	<i>Opuntia streptacantha</i>	Reference
∑Betacyanins	0.09–29.15 mg BE/100 mL	266–434 mg/100 g	–	0.24–1.38 mg BE/100 g	0.007 mg BE/100 g	0.16–30.05 mg BE/100 g	11.28 mg BE/100 g	Chavez-Santoscoy et al. (2009), Cassano et al. (2010), Del Socorro et al. (2015), Jiménez-Alvarado et al. (2015), Gouws et al. (2019) and Gómez-Covarrubias et al. (2020)
∑Betaxanthins	0.16–10.79 mg IE/100 mL	208–555 mg/100 g	–	0.31–0.44 mg IE/100 g	0.0005 mg IE/100 g	0.47–18.99 mg IE/100 g	6.63 mg IE/100 g	Chavez-Santoscoy et al. (2009), Cassano et al. (2010), Del Socorro et al. (2015), Jiménez-Alvarado et al. (2015), Gouws et al. (2019) and Gómez-Covarrubias et al. (2020)
∑Carotenoids	5.40–20.80 µg β-carotene/L	4.80–18.60 µg β-carotene/L	–	–	–	–	–	El Kharrassi et al. (2016)
DPPH-radical scavenging activity	1016.37–4368.50 µM TE/L	–	–	–	2.5, 400 µM TE/L	–	–	Zafra-Rojas et al. (2013), Del Socorro et al. (2015) and Castro-Muñoz et al. (2018)
IC <sub>50</sub> value (DPPH assay)	13.20 µL/mL	–	8.18 µL/mL	–	–	–	–	Ghazi et al. (2015)

BE betanin equivalent, CE catechin equivalent, GAE gallic acid equivalent, IE indicaxanthin equivalent, QE quercetin equivalent, TE Trolox equivalent

to eliminate stickiness in spray-dried fruit juice powders (Tonon et al., 2008). Firstly, Obón et al. (2008) investigated the feasibility of dried glucose syrup (DE 28–31) with a ratio of wall material: core material ranging from 0 to 3.33 for encapsulation of *Opuntia stricta* fruit juice by spray drying. The study's findings demonstrated that a minimum wall material: core material ratio of 1.66 and an inlet temperature of 160 °C are necessary to obtain free-flowing *Opuntia stricta* fruit juice powders with higher drying yield (60%) and the best color protection (Obón et al., 2008). The same researchers standardized the microencapsulation parameters to fabricate spray-dried powder colorant from *Opuntia stricta* fruit juice using glucose syrup (DE 28–31) as wall material and evaluated powders' stability in yoghurt and soft drink in their subsequent study. The study revealed that a wall material: core material ratio (°Brix/°Brix) higher than 1 enabled non-sticky powders' production. Additionally, higher juice concentrations (2.4 and 9.6°Brix) led to lower color strength, drying yield, and color yield than low juice concentration (1.2°Brix). The authors reported that a lower feed rate in spray dryer resulted in sticky powders, while particle size was reduced by increasing inlet air temperature (Fig. 40.5). Therefore, low juice concentration (1.2°Brix), high feed rate (0.72 L/h), and high inlet air temperature (160 °C) were determined as optimum process conditions. Optimized conditions yielded *Opuntia stricta* fruit juice powder with a betanin content of 357 mg/100 g. Throughout 1-month storage at room temperature, retention of optimized powder's color intensity was reported as 98%, which proved the benefit of the microencapsulation process on betalains' chemical stability. On the other hand, the color of *Opuntia stricta* fruit powder was more stable in yoghurt than that of soft drink owing to its higher pH value (pH 4.3) that is closer to the optimum pH value (4.5) of betanin stability (Obón et al., 2009).

MD is ranked first among the top wall materials due to its several advantages, including neutral flavor, cheapness, ability to form low-viscosity solutions, and to provide adequate protection against oxidation (Martínez et al., 2015). In their research, Castañeda-Yañez et al. (2018) evaluated three different types of MDs (Maprigel®0019, Maprigel®3204, Maprigel®4801) for the production of microcapsules containing pear juices of two *Opuntia* spp. varieties (red and purple) through spray drying. The use of Maprigel®0019 as wall material resulted in stickiness problems in the spray dryer. In all trials, lower drying temperature (120 °C) and feed flow rate (2.24 mL/min) improved the betalain retention rate and minimized the losses in DPPH radical scavenging activity color. In terms of wall materials, Maprigel®3204 served as a more suitable component for maintaining antioxidant activity and individual betalains in microcapsules.

Furthermore, regardless of wall material type and process conditions, microencapsulation provided better protection for juices of purple variety than that of the red one (Castañeda-Yañez et al., 2018). As an alternative to typical wall materials, dietary fibers have also been explored to encapsulate extracts of plant materials, oils, and pigments (Kurek et al., 2018; Kaderides & Goula, 2019; Sánchez-Madriral et al., 2019; Jiang et al., 2020). Codex Alimentarius Commission (2017) defines the dietary fiber as “carbohydrate polymers with 10 or more monomeric units that are



**Fig. 40.5** SEM images ( $\times 1000$ ) of powders produced from *Opuntia stricta* juices with a concentration of 12°Brix under different spray dryer conditions. (Adapted from Obón et al. (2009) with permission)

not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories:

- (a) Edible carbohydrate polymers naturally occurring in the food as consumed
- (b) Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities

- (c) Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

Dietary fibers may be categorized into four main groups as follows:

1. Low-molecular-weight dietary fibers (non-available oligosaccharides): inulin, fructooligosaccharides, galactooligosaccharides, polydextrose, resistant MD, raffinose, stachyose, verbascose, kestose, nystose
2. High-molecular weight dietary fibers (non-available homo-polysaccharides): resistant starches, cellulose,  $\beta$ -glucan
3. High-molecular weight dietary fibers (hetero-polysaccharides)
  - (a) Neutral hetero-polysaccharides: hemicellulose, pentosan, locust bean gum/galactomannan, guar, and Arabic gums
  - (b) Poly-electrolyte hetero-polysaccharides: pectin, carrageenan, alginate, chitin, chitosan, xanthan
4. Non-carbohydrate dietary fibers: lignin (Westenbrink et al., 2013).

The physiological effects of dietary fibers are the promotion of laxation, reduction of postprandial blood glucose and insulin responses, lowering of total and LDL cholesterol levels, acting as prebiotic and improvement of gut microbiota, enhancement of postprandial satiety, functioning as an antioxidant, and prevention of cancer, improvement of mineral absorption and reduction of metabolic syndrome symptoms (Alexandre & Miguel, 2008; Howlett et al., 2010; Fuller et al., 2016). Considering all these benefits, consumption of dietary fibers is recommended by several authorities, including World Health Organization (WHO), American Heart Association (AHA), Food and Drug Administration (FDA), and Australian National Health and Medical Research Council (NHMRC). Dietary fiber of rice bran, barley  $\beta$ -D-glucan, citrus pectin, inulin, and chia seed mucilage has been used for encapsulation of pear pulp, fish oil, corn oil, chia seed oil, probiotics, elderberry extract, and anthocyanins-rich extract of jaboticaba pomace up to now (Shah et al., 2016; Pereira Souza et al., 2017; de Campo et al., 2017; Castel et al., 2018; Kurek et al., 2018; Sobieralska & Kurek, 2019; Rios-Mera et al., 2019; Jiang et al., 2020). Accordingly,  $\beta$ -glucan (15, 22.50 and 30%, w/w) was evaluated as a wall material to convert the red-colored pear juice of *Opuntia ficus indica* into powdered form through a spray dryer having different inlet temperatures (160 °C, 180 °C, and 200 °C). As the fiber content increased, the microcapsules' size decreased as a result of the reduction of droplets' superficial tension. On the other hand, inlet temperatures above 160 °C led to great degradation rates in betacyanins (red/purple pigments). However, betaxanthins (yellow/orange pigments) were influenced by neither inlet temperature nor the amount of  $\beta$ -glucan. Taking into all results, the authors suggested the use of 22.5% of  $\beta$ -glucan and an inlet temperature of 160 °C for production of *Opuntia ficus indica* juice powder with the highest bioactives retention and improved physical attributes (Ruiz-Gutiérrez et al., 2014).



All wall materials have both advantages and disadvantages. None of them may meet all the properties required for the microencapsulation process. Thus, various combinations or mixtures of wall materials are preferred to fabricate microcapsules with better quality (Shahidi & Han, 1993). For this purpose, MD (DE 10) was used in combination with gelatin to encapsulate clarified purple-colored pear juice of *Opuntia stricta* by spray drying. In the study, different gelatin/MD ratios including 0:1, 0.25:0.75, 0.375:0.625, 0.25:0.75, 0.50:0.50 and 0.125:0.875 were used at different inlet temperatures (110 °C, 117.5 °C, 125 °C, 132.5 °C and 140 °C) with a fixed wall material:fruit juice ratio of 10%. Low encapsulation yields (7.76–14.8%) resulting from sticking of wall materials to spray dryer were reported, while betalain encapsulation efficiency values ranged from 18.1% to 57.3%. MD/gelatin combination led to the formation of agglomerated and firm-structured spherical particles without rupture. The betalain contents of microcapsules were influenced by both wall materials ratio and inlet temperature, whereas inlet temperature was the only determinant of the powders' DPPH radical scavenging activity. Based on the findings, the authors suggested using gelatin/MD combination at a ratio of 0.25:0.75 to obtain spray-dried *Opuntia stricta* fruit juice powders with high encapsulation yield and antioxidant activity (Castro-Muñoz et al., 2015).

### 3 Utilization of Microparticles Including Different Parts of *Opuntia* spp. in Food Products

Encapsulated products may be incorporated into various food and food-related products to improve their appearance, taste, flavor, nutritional quality, or storage stability. Mostly, microparticles are consisting of some parts or extracts of diverse *Opuntia* spp. plants have been employed as natural colorants due to their rich betalains contents. For instance, capsules of purple-colored *Opuntia ficus indica* pulp extract were incorporated into gummy candies as a source of betalains without significant color change at 4 °C for 30 days (Otálora et al., 2019). Accordingly, powders of red/purple-colored *Opuntia stricta* fruit juice and yellow/orange-colored *Opuntia ficus indica* pulp were successfully added to yogurt and soft-drink as natural food colorants (Obón et al., 2009; Carmona et al., 2021). On the other side, another research group developed edible starch films, including microcapsules of *Opuntia oligacantha* pulp extract with high phenolics, flavonoids contents, and total antioxidant activity (de Jesus Cenobio-Galindo et al., 2019).

### 4 Conclusion

Different parts of *Opuntia* spp. plants are excellent sources of various bioactives, particularly betalains. However, their high sensitivity against environmental conditions requires the application of suitable conservation techniques. Among these

methods, microencapsulation is considered the most promising one due to the utilization of various techniques and wall materials depending on the properties of core materials and the expectations from obtained microcapsules. Until now, most of the microencapsulation studies dealing with *Opuntia* spp. derived products or extracts have focused on using spray drying techniques due to their accessibility, simplicity, and cost-effectiveness. However, the process conditions of spray drying techniques such as inlet air temperature should be optimized in order to prevent thermal degradation of betalains, particularly betaxanthins. This problem may be solved using other encapsulation methods in which the products are not exposed to high temperatures during the process. Considering this aspect, freeze-drying can be served as a promising alternative to encapsulate heat-labile components. Encapsulated products of *Opuntia* spp. plants may be incorporated into different food products to bring unique colors to them. Therefore, food products become more attractive to consumers. Furthermore, rich bioactive contents of different *Opuntia* spp. parts may improve the nutritional quality and shelf stability of these products. In conclusion, encapsulation is a convenient way of preservation and different parts/extracts of *Opuntia* plants have been successfully encapsulated through this technique. Further studies are necessary to explore novel wall materials that provide the best protection for bioactive compounds of *Opuntia* spp.

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# Chapter 41

## Prickly Pear (*Opuntia* spp.) in Animal and Poultry Feed



Khalid M. Mahrose

**Abstract** Prickly pear is considered an excellent source for food supplementation, a multipurpose crop, and an alternative feed. Prickly pear is moderately high in sugars, starch, ether extract, crude protein, amino acids, fiber, and provides vitamins and calcium required in the animal diet. The nutritional and health benefits of prickly fruit are related to its antioxidant properties due to ascorbic acid, and phenolic compounds. Several studies showed that dietary prickly pear inclusion improves the feed digestibility and ammonia utilization through its conversion to protein.

**Keywords** Animals · Cactus pear · Performance · Egg · Meat quality · Antioxidative status · Immune response · Poultry · Rabbits

### 1 Introduction and Objectives

Prickly pear is considered as an excellent source for food supplementation (Feugang et al., 2006; Pinos-Rodriguez et al., 2007), as a multipurpose crop (Nazareno, 2017), and as an alternative feed (Bouzoubaâ et al., 2016; Makkar, 2017), due to its efficiency in converting water to dry matter, and thus to digestible energy balanced feed. It should rather be considered as a cheap source of energy (Nobel & Bobich, 2002). Prickly pear is moderately high in sugars, starch, ether extract, crude protein, amino acids, and fiber (Bhatt & Nagar, 2013; Osuna-Martinez et al., 2014; Makkar, 2017). Prickly pear provides vitamins and calcium that are required in the animal diet (Rodriguez-Garcia et al., 2007). It has been reported to have excellent DM digestibility (Gregory & Felke, 1992) and highly palatable to wild and domesticated rabbits (Hoffman et al., 1993). Prickly pear fruit is rich in betalains, carotenoids,  $\beta$ -carotene, ascorbic acid, and is one of the highest sources of total phenolic compounds (Ramadan & Mörseel, 2003; Yahia & Mondragon-Jacobo, 2011). The

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nutritional and health benefits of prickly fruit are related to its antioxidant properties due to ascorbic acid, phenolic compounds, and a mixture of yellow betaxanthin and red betacyanin pigments (Feugang et al., 2006). Previous investigations have suggested that the total antioxidant activity of prickly pear is due to its content of vitamin C, phenolics, flavonoid compounds (e.g., kaempferol, quercetin, and isorhamnetin), pigments (betalains), and taurine (Ramadan & Mörsel, 2003). Prickly pear pigments show important antioxidant activities without toxic effects. Meanwhile, the red cactus pears' free radical scavenging activity was related to the concentration of total phenolic compounds and ascorbic acid (Sumaya-Martínez et al., 2011).

This chapter reviews the effect of prickly pear and its derivatives on productive and reproductive performance, egg quality, antioxidative status, bacterial enumeration, and immune response of animals and poultry.

## 2 The Need for Phytogetic Additives in Animal and Poultry Feed

Phytogetic feed additives or phyto-additives are believed to be one of the greatest desirable replacements as growth and immune promoters (Salaheen et al., 2015; Hassan et al., 2016; Mahrose et al., 2019). Phytogetic feed supplementations could be defined as the complexes or combinations of botanical source integrated into poultry diets to improve productive, reproductive, and physiological performance as well as immune status and the quality of meat produced through the enhancement of feed properties, digestibility, nutrient absorption, and judgment of pathogens in the poultry gut (Athanasiadou et al., 2007; Windisch et al., 2008; Gadde et al., 2017; Ahmed et al., 2018a, b; Farghly et al., 2018; Madhupriya et al., 2018). Phytogetic feed supplementations have been shown to supply antimicrobial, antioxidative, immunogenic, and anticoccidial impacts when fed to poultry and rabbits (Hassan et al., 2016; Calik et al., 2019). Phytogetic enhances feed palatability, protects against oxidative damage, improve the gut health through reduced bacterial colony counts, declines fermentation products (including ammonia and biogenic amines), and decreases vigor of the gut-associated lymphatic order (Alagawany et al., 2018; Abd El-Hack et al., 2019; Abd El-Moneim and Sabic, 2019; Mahrose et al., 2019).

*Opuntia cladodes* are regarded as an excellent cheap feed source that can be used to reduce the cost of raising ruminant production in dry areas (Mokoboki & Sebola, 2017). Prickly or cactus pear [*Opuntia ficus indica* (L.) Mill.] is an alternate and low-cost forage crop along with the recent advancements in its cultivation and utilization as a food crop for supplementing the food security of a rapidly increasing populace (Iqbal et al., 2020). Cactus is a 'Bridge of life' for dry areas because it is a multipurpose plant and efficiently converts water into biomass (Tsega et al., 2016).

### 3 Effects of Prickly Pear and Its Derivatives On

#### 3.1 *Productive and Reproductive Performance of Animals*

Using dietary prickly pear fruit or prickly pear peel gave similar effects on growth performance traits as that of the control diet, as Hassan et al. (2019) indicated. Bakr (2019) reported that feed intake, protein intake, and digestible energy intake were reduced with rising prickly pear peel levels containing diets. The same authors found that rabbits fed diets supplemented with prickly pear peel showed feed conversion ratios. This indicates the potential of prickly pear fruit or prickly pear peel in alleviating feed and water lack (Amogne, 2007) and could be consumed successfully in the rabbit diets. Prickly pear is acceptable (Nefzaoui, 2017) and distinguished by high sugar substance (Feugang et al., 2006; Bouzoubaâ et al., 2016). Prickly pear is an abundant resource of water (80–95%), minerals, vitamins, and antioxidants as well as amino acids and fruit oils, while poor in crude protein, and most of the nitrogen is non-protein nitrogen (Feugang et al., 2006; Bhatt & Nagar, 2013; Osuna-Martinez et al., 2014; Makkar, 2017). Furthermore, prickly pear's high water content acts as a medium in nutrient carrying (Aguilar-Yáñez et al., 2011). These nutrients could hurry metabolism and boost energy digestibility and hence progressing growth performance. Though, the insignificant changes in growth performance, the findings of Hassan et al. (2019) recommend that comprising prickly pear in growing rabbit diets up to 50% denote advantages to the breeders by using minor quantities of intense feed. The latter authors attributed the equal performance of rabbits in their study to the similar chemical composition of the diets used and the insignificant differences in the digestion of all nutrients, nitrogen balance, and reduced  $\text{NH}_3\text{-N}$ . Zeedan et al. (2015) attributed the improved growth performance of rabbits fed prickly pear to prickly pear mode, including maintaining a beneficial microbial population and improving digestion. The same researchers added that prickly pear inclusion improves the feed digestibility and ammonia utilization through its conversion to protein. Ennouri et al. (2014) explained the improvement in growth performance of rabbits fed prickly pear by the activity of antioxidant, antimicrobial and anti-inflammatory compounds in prickly and nutrient utilization due to the presence of flavonoids and phenolic acids.

Prickly pear contributes to hot weather times as a lifesaving crop for animals (Gabriel et al., 2014). Ruminants generally eat 20–40 kg pads a day, although it could go up to 80 kg throughout the dehydrated periods for achieving the water requirement (Meraz-Maldonado et al., 2012). Holstein cows were observed to miss weight when prickly pear constructed 73% of their diurnal feed consumption. Other reports recommended substituting alfalfa hay up to 30% with prickly pear (Cordão et al., 2013). Small ruminants (sheep and goat), being nourished on prickly pear fodder, have also been observed to hurt from diarrhea due to a great level of minerals (Andrade-Montemayor et al., 2011). This trouble could be resolved by supplementing molasses in chopped prickly pear (Ben-Salem & Ennouri, 2013). Pinos-Rodriguez et al. (2007) revealed that dry matter intake and an average daily

weight gain of lambs were impacted and were lesser in the lambs consumed diet encompassing prickly pear forage than alfalfa hay. Ragab (2007) deduced that prickly pear peel could replace up to 30% of yellow corn in growing quail diets without any harmful impact on marketing body weight and body weight gain. The findings obtained by different researchers are vital in the expressions of sustainable animal production (Makkar, 2017), which presently is essential, and where the effective usage of accessible supplies is a primacy. Aranda-Osorio et al. (2008) and Aguilar-Yáñez et al. (2011) reported that the presence of 15–30% (DM basis) of prickly pear did not change the growth performance of lambs. Amogne (2007) found insignificant variations in body weight gain of lambs when prickly pear substituted 0, 20, 40, 60, and 80% of grassland hay. Meaningful outcomes were achieved in growth performance owing to consuming dietary prickly pear by Abu Shammalah (2007) and Zeedan et al. (2015). Mokoboki and Sebola (2017) showed that prickly pear should be modified for little crude protein and fiber matter Tswana goat feeding in Algeria. Also, the content of anti-nutritional factors should be verified as they do have an undesirable influence on goat performance. Abu Shammalah (2007) showed that rabbits fed diets comprising prickly pear had lesser final body weight and body weight gain than those fed the control diet. Islam et al. (2017) and Aware et al. (2017) stated that adding prickly pear in sheep and goat feeding has a favorable impact on body weight gain. A study was directed to examine the impact of consuming prickly pear (*Opuntia ficus indica*) fruit meal as a fractional substitute of corn on broilers' performance (Cobb 500). Tsega et al. (2016) concluded that the consumption of ash, crude protein, ether extract, and crude fiber were improved nonetheless; metabolizable energy was reduced with rising dietary concentrations of prickly pear fruit. However, daily weight gain of broilers was increased as increasing dietary prickly pear level. Further detailed investigations are required for deciding the influence of prickly pear fodder on all animal species performance in calls of milk, egg, wool, feather, and meat production.

### **3.2 Nutritional Evaluation of Prickly Pear, Digestibility Coefficients, and Nitrogen Balance**

Prickly pear is identified by the term forage palm and can be encouraged as a forage yield for dry Areas (Iqbal et al., 2020). The nutritional content of prickly pear differs according to several issues like age, season, weather, species, variety, soil type, fertility, and agronomic administration (Chimsa et al., 2013).

The principal constituents of prickly pear are carbohydrate-containing polymers, which encompass a blend of mucilage and pectin (Gabriel et al., 2014). López-García et al. (2001) showed that around 60% of the animals' total energy necessities could be provided by dietary prickly pear. Rodríguez-García et al. (2007) stated that the chemical arrangement of the pulp of prickly pear fruit was 91, 0.6, 0.1, 0.2, and 8.1 (g/100 g) for moisture, protein, lipids, fiber, and total sugar, respectively.

Likewise, Atef et al. (2013) reported that prickly pear fruit comprised 7.61% crude protein, 0.84% crude fiber, 3.88% ash, and 85.4% total carbohydrates. Guevara-Figueroa et al. (2010), Yahia and Mondragon-Jacobo (2011), and El-Mostafa et al. (2014) found that prickly pear peel and prickly pear fruit are abundant in vitamins A and E and free from alkaloids as anti-nutritional agents. Furthermore, Fernández-López et al. (2010) confirmed that the total phenol substance was 218.8 mg/100 g in prickly fruit pulp (*Opuntia ficus-indica*). Total antioxidant actions of differently colored prickly pear fruit quantified by dissimilar assesses were very associated with total phenolics, betalains, and ascorbic acid levels (Yahia & Mondragon-Jacobo, 2011). Gengatharan et al. (2015) reported that prickly pear has antioxidant, anti-lipidemic, and antimicrobial activities. Hassan et al. (2019) concluded that augmenting the level of dietary presence of prickly pear fruit, otherwise prickly pear peel, was related to decreased digestibility of the dry matter and augmented nitrogen-free extract. The latter researchers added that the other nutrients' digestibility was insignificantly varied by prickly pear fruit and prickly pear peel as well. The same authors indicated that prickly pear fruit and prickly pear peel dietary concentrations did not impact the nitrogen balance in the experimental groups. Pinos-Rodriguez et al. (2007) revealed that organic matter *in vivo* digestibility was more significant with prickly pear than alfalfa, but N balance was harmfully influenced by prickly pear. On the other hand, Ben Salem et al. (1996) and Zeeman (2005) indicated that due to the great substance of effortlessly digestible carbohydrates of prickly pear, its dry matter digestibility was raised. The elevated concentration of effortlessly digestible carbohydrates in prickly pear boosts nitrogen conversion into microbial protein (Zeedan et al., 2015). Amogne (2007) declared that lambs' digestibility was impacted by the nutritional presence of prickly pear in lamb diets up to 80% as a substitute for grassland hay.

### 3.3 Carcass Traits and Meat Quality

The assessment of carcass quality of animals and poultry species is an extremely crucial part of the production and advertising of products (Tsega et al., 2016). Carcass traits are the critical issue to appraise the quality of the carcass. In an experiment on growing New Zealand white rabbits, Hassan et al. (2019) concluded that the insignificant changes in carcass traits might be assigned to the insignificant variations in the digestibilities of most of the nutrients in their work. Increasing liver weight in rabbits fed diets containing 50% PPF or PPP may be attributed to the liver's involvement in metabolism (Abu Shammalah, 2007). The later author reported that the diet containing prickly pear increased liver weight and the other edible parts of rabbits. It is essential to point out that dietary prickly pear peel reduced abdominal fat percentage and improved carcass weight. Aguilar-Yáñez et al. (2011) showed insignificant differences in lamb carcass characteristics due to dietary prickly pear. In the same line, Mahouachi et al. (2012) concluded that carcass weight of goat was not significantly influenced by dietary prickly pear. On the

other hand, Zeedan et al. (2015) indicated that rabbits fed a diet containing 30% of prickly pear had the highest values of all carcass characteristics due to increased growth performance. The latter authors added that rabbits fed diet containing prickly pear had lower abdominal fat than the control group and attributed the abdominal fat reduction to the lower digestible energy intake by rabbits fed prickly pear.

In broiler chicks, Tsega et al. (2016) pointed out that broiler chicks consumed various prickly pear fruit meals with greater carcass quality as determined in terms of thigh, drumstick, and breast muscle. The same authors stated that substitution of approximately 6.75% of corn with prickly pear fruit meal in broiler diets has no adverse consequences on thighs and drumsticks weight. This implies that in regions where corn is not obtainable, prickly pear fruit could be consumed as an energy supplier, particularly under a minor scale production method. Feeding broiler chicks on dietary prickly pear led to the deep yellow coloration of shank, beak, skin, and fat, and that may be attributed to the existence of natural suppliers of xanthophyll found in prickly pear (Tsega et al., 2016).

Carcasses of commercial Cobb chickens had higher crude protein content and lower fat than that of control when birds fed diets containing 15% prickly pear (*Opuntia ficus indica*) peel powder replaced with yellow corn grains (Badr et al., 2019). On the other hand, Ragab (2007, 2012) indicated that the dietary level of prickly pear insignificantly changed broiler or quail meat's chemical composition.

### 3.4 Blood Biochemicals and Antioxidant Status

In a trial conducted by Njku et al. (2018), Wistar rats were fed on 100, 300, and 500 mg/kg of matured stem of *Opuntia dillenii* aqueous extract. The authors found a rise in alanine aminotransferase activities and alkaline phosphatase in rats fed 100 and 300 mg/kg when compared with the control. The authors attributed that increase as a consequence of irritation and disorders of hepatocytes penetrability. The latter researchers stated that rats consumed 300 mg/kg showed a reduction in urea and creatinine levels. A decline in triglyceride, total cholesterol, and low-density lipoprotein cholesterol, was observed in rats fed 100 and 300 mg/kg, due to the hypocholesterolemic abilities of the developed stems of *Opuntia dillenii*. The same investigators found that the hematological assessment explored a reduction in red blood cell and hemoglobin levels in rats fed 300 mg/kg when evaluated with their counterparts. Urea, created by the liver and the chief nitrogenous termination result of the amino acid analysis, continues the greatest frequently exploited medical directories for renal job appraisal (El-Said et al., 2011). Creatinine, a catabolic result of creatinine phosphate in muscles, is frequently benefited to evaluate kidney job, and its comparative making by the body mass is reliant on the mass of the muscle (Zuo et al., 2008). Njku et al. (2018) proposed that aqueous extract of *Opuntia dillenii* does not converse nephrotoxic impacts, so urea concentration was reduced in their work. In broilers, glucose, triglycerides, and cholesterol levels were lesser in the blood of broilers consumed diet incorporated with 10% of *opuntia ficus*



compared to the control group (Moula et al., 2019). However, the latter authors found that uremia's dosage was significantly greater in birds fed diet supplemented with 5% of *opuntia ficus*, compared to the control group. However, no significant difference was detected in total proteins. The same authors attributed the hypoglycaemic impact of cactus to its wealth in dietary fiber, principally pectin, which triggers a reduction in the captivation of carbohydrates by creating a pectin gel.

Prickly pear has motivated antioxidant activity and protective capability due to several compounds like vitamins E and C, phenolic compounds, and other non-nutritional substances (Ramadan & Mörsel, 2003; Yahia & Mondragon-Jacobo, 2011). Phenolic compounds have been reported in prickly pear as an antioxidative due to the main flavonoids encountered (Feugang et al., 2006; Saih et al., 2017). These compounds are more effective antioxidants than vitamins since they can delay prooxidative impacts on proteins, DNA, and lipids by generating stable radicals (Shahidi et al., 1992). Furthermore, when prickly fruits are subjected to study, it must be taken into consideration that higher phenolic compounds are found in the peel rather than the pulp (Feugang et al., 2006). Hence, from a nutritional point of view, processing both fruit and pulp appears beneficial to the rabbits breeder.

Reactive oxygen species (ROS) extend a multiplicity of vital impacts through a wide range that includes physiological regulatory functions and damaging alterations involved in the pathogenesis of an increasing number of diseases (Osuna-Martinez et al., 2014; Saih et al., 2017). The additive and synergistic effects of phytochemicals in prickly pear are responsible for its antioxidants activities, and that the benefits of prickly pear-based diets are in part due to the complex mixture of useful compounds present in prickly pear. The antioxidative ability of the prickly pear could neutralize ROS (Feugang et al., 2006). All prickly pear levels reduced triglycerides, cholesterol, and LDL, while increased HDL. The reduction in cholesterol concentration may be related to the inclusion of dietary prickly pear. Prickly pear contains pectin, which interferes with cholesterol and lipids synthesis through binding cholesterol to bile acids (Louacini et al., 2012; Zeedan et al., 2015; Nazareno, 2017) and then when the concentrations of these compounds increase, they accelerate the catabolism of cholesterol (Louacini et al., 2012). The interaction among flavonoids, betalains, and vitamin E of prickly pear seems to give it hypolipidemic activity (Lee & Lim, 2000). Similar results were obtained by Zeedan et al. (2015). Aguilar-Yáñez et al. (2011) demonstrated that dietary prickly pear reduces cholesterol and triglycerides in the bloodstream.

### 3.5 Intestine, Rumen, and Caecum Parameters

In their experiment on growing rabbits, Hassan et al. (2019) claimed that caecum pH value did not alter attributable to nutritional insertion of prickly pear fruit and prickly pear peel. The same authors also added that rabbits ate diet encompassed 50% of prickly pear peel had the lowermost assessment of  $\text{NH}_3\text{-N}$  as matched with their counterparts. Additionally, rabbits ate prickly pear fruit or prickly pear peel

had lesser means of volatile fatty acids than the control group. Caecum pH is of greatest imperative measure that influences microbial fermentation and caecum roles. Prickly pear enhances the intestine's circumstance and hurries the digestibility (Zeedan et al., 2015). The pH worth of rabbit cecal denotes a dropping propensity when total volatile fatty acids levels develop and ammonia level decreases (Garcia et al., 2002). Pinos-Rodriguez et al. (2007) showed that Ruminant pH was lesser when lambs ate prickly pear matched with alfalfa as fodder. Acetate, propionate, butyrate, total volatile fatty acids, and acetate: propionate ratio were comparable with prickly pear or alfalfa. Nonetheless, Hassan et al. (2019) attributed the absence of difference in cecal pH value in their study to the enhancements in cecal physico-chemical circumstances once prickly pear was supplemented with prickly pear fruit or prickly pear peel.

Numerous reports suggest that prickly pear have a short retaining time in the rumen, little proportion of fiber, and great content of soluble carbohydrates quickly fermentable in the rumen (Nefzaoui & Ben Salem, 2002; V eras et al., 2005). These prickly pear characters may decrease ruminal pH and bacterial cellulolytic activity (Pinos-Rodriguez et al., 2007).

Rabbits consumed diets encompassing 50% prickly pear peel had the lowermost  $\text{NH}_3\text{-N}$ , and that indicates expanding the acidity then the action of microbial production located in the caecum (Hassan et al., 2019). Prickly pear is a gorgeous supplier of amino acids, and that could help rabbits to exploit them and to reduce  $\text{NH}_3\text{-N}$  than those of the control group. Hereafter, the propensity to lesser  $\text{NH}_3\text{-N}$  levels could be accredited to superior ammonia consumption by cecal microbes. Rabbits are distinguished by night caecotrophy, in which rabbits re-ingest the excreta and use the microbial protein. Zeedan et al. (2015) indicated that expanding dietary prickly pear up to 30% is directed to a reduction in  $\text{NH}_3\text{-N}$  compared to the control group. Rabbits consumed 25 and 50% of a prickly pear fruit, and 25% of prickly pear peel caused fewer caecum fermentation. Caecum fermentation creates total volatile fatty acids, accounting for 40% of the rabbits' calorie necessity (Marty & Vernay, 1984). Volatile fatty acids likewise help influence pathogenic bacteria by aiding in preserving the normal pH (6–7) in the caecum (Proh aszka & Szemer edi, 1984; Fortun-Lamothe & Boullier, 2007). The dietary incorporation of prickly pear in sheep allows superior captivation of total volatile fatty acids (Cordova-Torres et al., 2017). Outstanding to the reality that the tough fraction of prickly pear is exceptionally digestible, at concentrations upper than 40% of prickly pear, total volatile fatty acids concentration is being high (Lebas et al., 1986). Misra et al. (2006) suggested that prickly pear's escalating presence in lamb diets up to 40% directed to an escalation in total volatile fatty acids.

## 4 Conclusion

Diets including prickly pear had constructive influences on productive and reproductive issues of animals and poultry species, which makes it a value and sustainable feeding approach, especially within a maintainable animal production term. This could be appealing to the breeders of animals, since the prickly pear is a plant that grows over the year, and can be used as herbage resource, mainly in parts where herbage production is inadequate.

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# Chapter 42

## Industrial Applications of *Opuntia* spp. (Nopal, Fruit and Peel)



Tukayi Kudanga and Christiana Elejo Aruwa

**Abstract** Research interest in the *Opuntia* species has been increased, especially in the past decade. Although it was previously thought to be a weed menace and underutilised, the *Opuntia* plant possesses several food and non-food applications. This chapter reviews the industrial applications of *Opuntia* spp. and the real prospects that could guide future research directions. In food applications, *Opuntia* can be used as feed/fodder, nutraceuticals, beverages, sweeteners, and food additives. Non-food applications have broadened to include the textile, fuel, bioplastic, wastewater treatment, pharmaceutical, medical, cosmeceutical, agrochemical, agroforestry, and pollution control industries. *Opuntia* aerial parts such as cladodes, fruits and peel by-products are rich in nutrients and phytochemicals, many of which are yet to be profiled and identified. It is therefore envisaged that biotechnological applications of the plant will continue to increase. Although the *Opuntia* plant has shown promise for several industries, many *Opuntia*-based products have not yet been commercialised. Factors contributing to this challenge are highlighted, and possible workable solutions suggested.

**Keywords** *Opuntia* spp. · Cladodes · Fruits · Peels · Non-food application · Commercialisation · Food applications

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## Abbreviations

Al	Aluminium
Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	Aluminium sulphate
ANADEC	National Association of Cactus Development
CactusNet	Cactus Network
CAM	Crassulacean Acid Metabolism
CO <sub>2</sub>	Carbon dioxide
COD	Chemical Oxygen Demand
CODEX STAN	Codex Alimentarius Standard
COX	Cyclooxygenase
CSJ	Cactus and Succulent Journal
CSSG	Cactus and Succulent Specialist Group
FAO	Food and Agricultural Organisation
Fe	Iron
GMP	Good Manufacturing Practices
GRIN	Germplasm Resources Information Network
GSH	Glutathione
HACCP	Hazard Analysis and Critical Control Point
HCl	Hydrochloric acid
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JPACD	Journal of the Professional Association for Cactus Development
LAB	Lactic Acid Bacteria
MAs	Macromolecular Antioxidants
MDA	Malondialdehyde
Mn	Manganese
QA	Quality Assurance
QC	Quality Control
R&D	Research and Development
SAERT	Sustainable Agriculture and Environmental Rehabilitation in Tigray
SOPs	Standard Operating Procedures
TSS	Total Soluble Solids
UVB	Ultraviolet B
WHO	World Health Organisation

## 1 Introduction

The cactus pear (*Opuntia*) plant dates back to the Aztecs in the Mesoamerican region (Pimienta, 1990). The Spanish people consider the *Opuntia* plant an all year round delicacy to be relished, while nomads considered proximity to the cactus pear plant a reason to take up settlements (Bravo-Hollis, 2002). The cactus pear fruit value was also equated to the value of silver and gold in the western Indies since a

vast number of ethnomedicinal properties were alluded to members of the *Opuntia* species (Velásquez, 1998). The *Opuntia* plant is an integral part of the Mesoamerican history and contributes to the people's socio-economic life (Flores-Valdez, 2003). Either cultivated or wild, the *Opuntia* species grow across various agricultural conditions and climates where they serve as raw materials for various industries and as food. As a result, the plant has since spread from the Americas to regions and continents such as Asia (India), Africa (Angola, Tunisia, Nigeria, Algeria, Ethiopia, Egypt, South Africa, Eritrea, Morocco, Libya), Australia, Europe, the Caribbean and Mediterranean regions (FAO, 2013). Some of the wild *Opuntia* varieties include *Opuntia leucotricha*, *Opuntia robusta*, and *Opuntia hyptiacantha*, while the most cultivated *Opuntia* species are the *Opuntia streptacantha*, *Opuntia xocoonstle*, *Opuntia amyclae*, *Opuntia megacantha*, and *Opuntia ficus-indica*. However, globally the *Opuntia ficus-indica* is the most widely cultivated (Uzun, 1996).

The *Opuntia* plant shows significant taxonomic complexity with significant variations in species and phenotypes across different environments, locations, and climates. It is classified within the Cactaceae family and reproduces either asexually or sexually (GRIN, 2005; Aruwa et al., 2018). The name 'nopal' refers to the entire cactus plant; the fruit is called 'tuna', the tender cladodes 'nopalitas', and the mature fleshy leaves or cladodes called 'penca', all of the Spanish nomenclature. Israelis call it 'sabras' meaning 'prickly on the outside but sweet on the inside'. It is mainly used as livestock forage in Brazil and was therefore named 'palma forrageira'. The plant's morphological and anatomical characteristics enhance its adaptability to various environments. This attribute makes the plant a valuable natural resource that cannot be overlooked in many ecological zones for its socioeconomic potential (FAO, 2013).

The cladodes are also scientifically referred to as stems, racquets, fleshy leaves, or paddles. Cladodes may or may not have spines. Cladodes function as thick cuticle bound leaves with a waxy covering (or glochids) that decrease water loss. The presence of numerous parenchyma enables its high water storage capacity. Mucilage, a hydrocolloid present in the paddle tissues, also has a tremendous capacity to bind water molecules (Nobel et al., 1992). Cladodes have stomata across the surface area, which prevent moisture loss and absorb carbon dioxide (CO<sub>2</sub>) at night for photosynthetic roles. Its peculiar photosynthetic function is of the crassulacean acid metabolism (CAM), where stomata open at night to take in CO<sub>2</sub>, which then gradually acidifies the paddles and stem (Díaz et al., 2017). Plants capable of CAM are also reported to resist high and low temperatures favourably (Nobel, 1998). Even in extreme droughts, cladodes are only slowly dehydrated and degraded. These characteristics increase the *Opuntia* plant's economic and agricultural viability across semi-arid and arid terrains, where little or no irrigation platform is required (Nobel, 1998; FAO, 2013).

Given the characteristics mentioned above, the economic potential of the *Opuntia* plant and cladodes is increasing. Table 42.1 summarises the food and non-food applications of various parts of the plant. These are further elaborated in respective sections. The chapter concludes with an overview of the factors affecting the commercialisation of *Opuntia*-based products.

**Table 42.1** *Opuntia* plant parts and their food and non-food applications

S/N	<i>Opuntia</i> plant part	Product(s)	Industry application	Reference
<i>Food application(s)</i>				
1	Nopals and cladodes	Forage or feed	Agriculture	FAO (2001) and Fuentes (1991)
		Flour	Food (cooking, baking, maltodextrin, confectioneries)	FAO (2013)
		Jams, Sauces, Juices, Pickles	Food	FAO (2013)
		Hydrocolloids (mucilage)	Food (thickening agents)	Sáenz (2004)
		Mucilage or gum	Food (fruit leathers or edible films)	FAO (2013) and Sepúlveda et al. (2003)
		Cereals	Food (cereal and nopal flour mix; pelleted cereal products)	FAO (2013)
		Nopalitos in chilli sauce, Azteca, Nopal mixed with guava juice (export product)	Food	FAO (2013)
		'Agua de Nopal' also known as nopal water (cladode juice and sugar)	Food	FAO (2013)
2	Fruits	Beverages Syrup, Toffee (melcocha), and Cheese. Nectars, Gels, Juices, Jams, Liquors, Juice concentrates. Sweeteners, Vinegars, Wines and Brandies, Canned fruits, Frozen pulp, and other fruit products Functional foods (nutraceuticals)	Food	López et al. (1997), Corrales and Flores (2003), FAO (2013), Corrales and Flores (2003), Sáenz (2000) and Sloan (2000)
		Edible oils or fatty acids	Food and feed supplement	Ennouri et al. (2006) and Sawaya et al. (1983)
		Cocoa butter	Food (chocolate productions)	Jana (2012)
3	Peels	Thickeners, Prebiotic preparations	Food	Díaz-Vela et al. (2013)
		Sauces	Food	Sáenz (1999)

(continued)

**Table 42.1** (continued)

S/N	<i>Opuntia</i> plant part	Product(s)	Industry application	Reference
		Peel powder	Food (flour supplementation in bread baking, baked products improvers; substitutes for sugar, vitamin E, and fats)	Anwar and Sallam (2016) and Chougui et al. (2015)
<i>Non-food application(s)</i>				
1	Nopals and cladodes	Carbon sink	Ecosystem conservation (CO <sub>2</sub> removal or reduction)	Nobel and Bobich (2002)
		Carmine and grana	Paper, Ceramic, Textile, Cosmetic and Food	Aldama-Aguilera et al. (2005) and FAO/WHO (2000)
		Biogas and other fuels Carbon dioxide production Ethanol	Energy (cooking fuel, heating, electricity)	Aké Madera (2018), Ciriminna et al. (2019), Tegegne (2002), Varnero and García de Cortázar (1998), García de Cortázar and Varnero (1999) and Stintzing and Carle (2005)
		Capsules and tablets (for diabetes and obesity management) Gastric mucosal protectants Other potential pharmacological products	Pharmaceutical and medical	Fрати-Munari et al. (1992), Trejo-González et al. (1996), FAO (2013), Aruwa et al. (2018), Díaz et al. (2017), Park et al. (2017) and Viegi et al. (2003)
		Shampoos, Gels, Creams, Lotions	Cosmetic	Corrales and Flores (2003)
		Natural additives	Cosmetic, Pharmaceutical, Food	FAO (2013)
		Binding agents Anti-corrosives	Construction (adherents, monument restoration, etc.)	Hammouch et al. (2004) and Torres-Acosta et al. (2005)
		Artisan crafts (baskets, bangles, earrings, artifacts)	Tourism	FAO (2013)
		<i>Opuntia</i> hydrocolloids or mucilage	Environment and water (water and wastewater treatment)	Miller et al. (2008) and Sáenz et al. (2004)
		<i>Opuntia</i> and its composted products	Agriculture (hedge, biofertiliser)	FAO (2013) and García (1994)
		Insect repellent	Ethnomedicinal	FAO (2013)
		Nopalitos and cattle manure vermicompost	Agriculture (vermiculture)	García (1994)

(continued)

**Table 42.1** (continued)

S/N	<i>Opuntia</i> plant part	Product(s)	Industry application	Reference
		Drought protectant	Agriculture and conservation	FAO (2013)
		Gramophone needles	Music	Ramsay (1928)
		Biomaterials and bioplastics	Biomedicine and packaging materials production	López-Palacios et al. (2016)
2	Fruits	Pigments and colorants (natural additives)	Many industries	FAO (2013)
		Fruit fibres Other potential pharmacological products Nutrient supplements	Pharmaceutical (health and wellness)	FAO (2013), Hollingsworth (1996), Hahm et al. (2015), Serra et al. (2013), Chahdoura et al. (2015) and Ghazi et al. (2013)
		Fruit seed oils	Construction (anti-corrosion)	Hmamou et al. (2012)
		Dried or processed fruit by-products (seed)	Agriculture (food and feed supplement)	Sawaya et al. (1983)
		Cocoa butter	Pharmaceutical, Cosmetic and Toiletry production	Jana (2012)
3	Peels	Betainin (betalain), xanthophyll, and other pigments	Many industries (natural additives)	Abou-Elella and Ali (2014) and Cano et al. (2017)
		Biofertiliser	Agriculture	Quintanar-Orozco et al. (2018)
		Biogas and other fuels	Energy	Gebrekidan et al. (2014) and Quintanar-Orozco et al. (2018)
		Dried peel supplements, peel extracts and other potential pharmaceuticals	Pharmaceutical (pharmaceuticals for health and wellness)	Wiese et al. (2004), Abou-Elella and Ali (2014), Cerezal and Duarte (2005), Federici et al. (2009) and Milán-Noris et al. (2016)
		Bioprotein (additive)	Chemical and pharmaceutical	Gad et al. (2010)

## 2 Food Applications of the *Opuntia* Cladodes

Not many plant species are as versatile as the *Opuntia* with regard to processing into foods and food products. In particular, cladodes have been processed into many food products and have also been used as forage and feed.

## 2.1 Forage and Feed

The *Opuntia ficus-indica* cladodes are consumed as food and vegetables by humans and also serve as forage for livestock in locations like Mexico (Flores & Aguirre, 1979; Fuentes, 1991), Chile and the United States (Hanselka & Paschal, 1990), and Brazil (Domingues, 1963). Exploration for forage uses has also been reported in South Africa (Wessels, 1988) and North Africa (Monjauze & Le Houérou, 1965).

The use of *Opuntia* spp. as feed is closely linked to its ability to efficiently convert water to digestible energy and dry matter (Nobel, 1998). Another unique advantage of the plant is that in semi-arid and arid conditions, the *Opuntia* or CAM species play a superior role in the amount of dry matter converted per surface area than other broadleaves and grasses used as feed plants. The *Opuntia* plant and cladode are useful as forage for pigs and cattle but have to be mixed with other foods rich in proteins since *Opuntia* spp. are low in protein content (FAO, 2001). Since the cladodes have a high water holding capacity, they are a good feed source for cattle, especially during droughts (SAERT, 1994). Spiny and slow-growing *Opuntia* cladodes and cultivars do not need to be protected from herbivorous animals like the spineless cultivars. However, when using prickly varieties as feed, the spines have to be removed or burned off before utilisation as feed for livestock. About 100,000 ha and 900,000 ha of land have been dedicated to *Opuntia* cultivation for fruit production and forage, respectively, in countries worldwide (FAO, 2001). Thus, the use of *Opuntia* species as forage or feed still outweighs other applications.

## 2.2 Food Products

Examples of food products derived from *Opuntia* cladodes include jams, sauces, juices, and pickles (Table 42.1). Other products that require minimal processing are also possible. Cladodes may also be useful for processing into flour. At the age of 2 to 3 years, cladodes are partially lignified. In this form, they can be processed into flour for use in cooking and baking. The flour can also serve as food, a feed supplement, and an essential ingredient for the preparation of confectioneries (FAO, 2013).

The presence of mucilage and hydrocolloids in nopals makes them useful in producing thickeners (Sáenz, 2004). The mucilage or gum derived from the *Opuntia* have been shown to demonstrate specific properties. Such properties include the ability to maintain a system's viscosity while preventing system flocculation, to reduce surface tension and improve emulsion stabilisation (for dairy products and non-alcoholic beverages), and to stabilise foams (Garti, 1999). The use of mucilage in fruit leathers and edible film production, product texture development, syneresis inhibition, and crystallisation control has also been reported (FAO, 2013). However, fruit leathers have not been produced on an industrial scale (Sepúlveda et al., 2003).

New food product varieties that are *Opuntia* cladode-based have been produced in Mexico. They are a combination of tender cladodes and other ingredients and

include cereal and nopal flour mixtures; pelleted cereal products from nopal powder, maltodextrin, wheat bran, and flour; tender nopalitos in hot chili (*Capsicum annuum*) sauce; Azteca, a nopalitos salad incorporating tuna fish; and a nopalitos puree or pâté with soybean and beef or chicken flavouring. A nopal juice product, which is combined with guava juice, is also commercially available for domestic markets and exportation. Another product is 'Agua de Nopal' meaning cladode water, and is composed of nopalitos juice and sugar. Pickled tender cladodes and crystallised and candied nopalitos products have also been produced (FAO, 2013).

### 3 Non-Food Applications of *Opuntia* Nopals/Cladodes

The industrial potential of the *Opuntia* plant extends beyond food applications. Some of the non-food applications of *Opuntia* are discussed in this section.

#### 3.1 Carbon Sink

Given the rise in fossil fuel usage, deforestation, and the subsequent increased levels of atmospheric CO<sub>2</sub>, the earth's ecosystem faces a major challenge. Through its paddles, the *Opuntia* plant could serve as an absorbent for the removal of excess carbon dioxide in regions that may be suitable for the establishment of the plant, which sometimes are unfavourable for other plant species. In this scenario, the plant acts as a carbon sink (Nobel & Bobich, 2002).

#### 3.2 Grana/Carmine Production

Some *Opuntia* species, such as the *Opuntia cochenillifera* and *Opuntia ficus-indica*, are specifically chosen for rearing female cochineal insects (*Dactylopius coccus*) for grana and carmine (an industrial colourant) production. To date, of several *Opuntia* species, only a few have been explored for grana or cochineal production on a large scale (Uzun, 1996). Grana or carmine produced from cochineal insects are natural colorants used in the paper, ceramic, textile, cosmetic, and food industries. Laboratory dyes are also prepared from cochineal products. Carmine, in its various marketed forms (carmine, carminic acid or carminic solutions), has been added by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to the authorised food additives list of the Food and Agricultural Organisation (FAO) (FAO/WHO, 2000). Peru leads the international exportation market for dried cochineal products. Globally, the export value for carmine or grana stands at \$60 to \$112 per kilogram of dried carmine. With increased awareness about this natural

colorant, global demand is expected to increase (Aldama-Aguilera et al., 2005). Natural pigments serve as excellent and favourable alternatives to synthetic colourants.

### 3.3 Fuel and Bioenergy Generation

When cladodes become completely hardened or lignified, they may be burned as fuel. Cladodes, thus, play a role in the energy sector. As cladodes age, the fiber content increases to about 16.1% in the lignified stems (Flores et al., 1995), and crude fiber content as high as 17.5% in lignified cladodes has been recorded (Flores-Valdez, 1999). Tegegne (2002) reported higher ash content in lignified cladodes compared to younger paddles. This property makes the use of lignified cladodes as fuels possible.

The digestion of nopals with factory waste results in the production of biogas for cooking and other uses. Nopal and manure mixtures at various ratios and under different conditions produce a variety of biogases when fermented. Carbon dioxide is the major gas generated at a pH  $< 5.5$  such that the gas's energy content and combustibility is reduced. Biogas with a 60% methane content was also generated at a pH  $\geq 6$ . Biogas composition from fermentation is closely linked to the raw materials' pH (Varnero & García de Cortázar, 1998).

More recent reports have shown that compared to jatropha, corn, palm, and sugar cane plant biomasses, the nopal biomass shows great economic feasibility in its ability to generate biogas. The *Opuntia* nopal utilises less biomass to generate more biogas (Kleiner, 2018; Ciriminna et al., 2019). In Chile, a small scale biogas plant started operations and highlighted some identifiable technical considerations linked to biogas production from the *Opuntia* nopal. Some of these include the rapid degradation of nopals compared to manure, and fermentation takes place within an acidic range of 6.5 to 6.8 and at room temperature. It can be produced using a bioreactor with an epoxy resin internal coating (economically viable and does not require stainless steel). The fermentation wastewater was also rich in nitrogen and was useful in plantation irrigation. The fermentation residue or solid fibre could also be directly used as a fertiliser or included in compost (Kleiner, 2018). A company in Mexico, NopaliMex, has also successfully used *Opuntia* cladode-derived biogas in running its tortilla production processes. The gas met the company's electricity and heating and fuel needs (Aké Madera, 2018).

Ethanol production follows a more complex procedure and usually requires specific yeast cultures for maximum ethanol generation (García de Cortázar & Varnero, 1999). Bioethanol generation has been recorded through cladode fermentation following prior treatment with acid (hydrochloric acid), and enzyme (cellulase) hydrolysis. These pre-treatments served for the release of saccharides required for *Saccharomyces* sp. fermentation. A yield of 9 L was derived from a 100 kg cladode biomass. Again, an average of 3000 L from irrigated and 300 L from non-irrigated lands could be produced from a plant density of 635 to 5000 per hectare (Retamal et al., 1987; Stintzing & Carle, 2005).

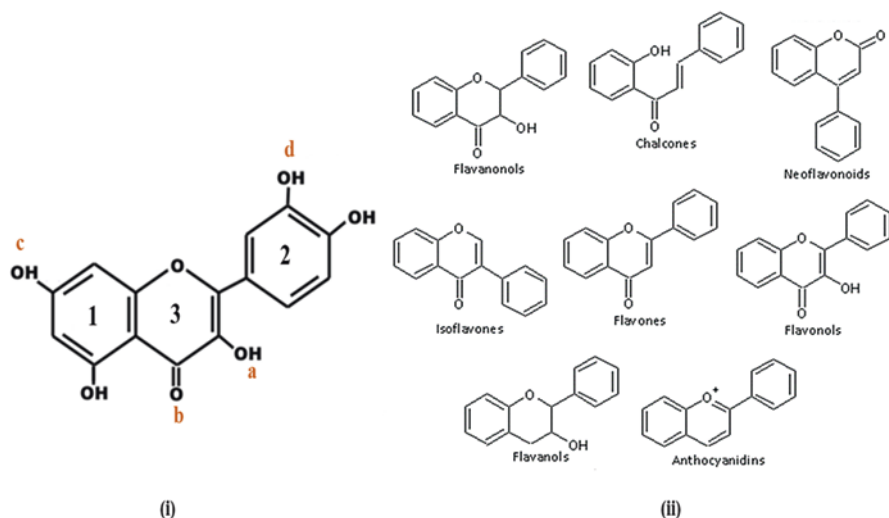


### 3.4 *Pharmaceutical and Medical Applications*

Cladode fibre has been made into capsules or tablets for diabetes and obesity control. Capsules made from *Opuntia streptacantha* (Frati-Munari et al., 1992) and *Opuntia fuliginosa* (Trejo-González et al., 1996) showed the most effective hypoglycaemic action compared to *O. ficus-indica*. Mucilage extracts have also been used to make gastric mucosal protectants. Other potential pharmacological effects reported for cladode extracts include antioxidant, antibacterial (Lee et al., 2002a; Aruwa et al., 2019a, b), antiviral (Ahamd et al., 1996), anti-inflammatory (Galati et al., 2002), analgesic (Park et al., 1998), and antiulcerogenic (Lee et al., 2002b; Galati et al., 2002) effects. Other researchers reported anti-atherogenic (Budinsky et al., 2001) and anti-spermatogenic (Gupta et al., 2002) properties.

New possibilities abound for the identification of pharmaceutical products based on nopalito extracts and compounds. An array of biomedical applications are possible, including use in chronic disease (cancer, obesity and diabetes, cardiovascular and atherosclerosis diseases) therapy (Osuna-Martínez et al., 2014; Díaz et al., 2017). Nopalito powder has been used to increase fibre intake and in blood sugar and weight management. Rehydrated powder gels are used in wound healing due to the cooling effect. Soluble nopal fibre extracts improved peristaltic movement in the colon, which impacted stool bulking and passage, while insoluble cladode fibres are reported to bind toxins (Stintzing & Carle, 2005). *Opuntia* species like *O. streptacantha* and *O. fuliginosa* have been implicated in liver injury, fatigue, gastritis, and dyspnoea therapies in ethnomedicinal applications (Hitchcock et al., 1997; Shapiro & Gong, 2002a, 2002b). In Italy, *O. ficus-indica* ethnoveterinary use has been reported (Viegi et al., 2003). Cladodes have also been used in arteriosclerosis and acidosis treatment (Warschkow & Warschkow, 1994; Hegwood, 1990). The liver protective activity has also been recorded for cladode extracts (Hfaiedh et al., 2008). Antioxidant and antidiabetic extract activities were linked to the presence of lignans, polysaccharides, phenolic acids, and flavonoids (Fig. 42.1). Various substitution patterns in medicinal plants are possible to yield different classes of flavonoids, such as flavones and flavanols (Rocchetti et al., 2018).  $\alpha$ -Pyrones and the new opuntioside and opuntiol moieties with analgesic activity have also been identified in *O. dillenii* cladode extracts (Siddiqui et al., 2016). Protection against ultraviolet B (UVB) skin damage is also a potential use for *O. humifisa* pad extracts (Park et al., 2017).

The identified pharmacological effects of *Opuntia* extracts and compounds are closely linked to an array of action mechanisms. Pharmacological activities such as cardio-protection and antioxidant activities attributed to flavonoid compound groups have to do with redox-active metal chelation and lipid peroxidation inhibition mechanisms. These mechanisms involve the transfer of electrons (Tsao, 2010). Other modes of action include oxidase inhibition and antioxidant enzyme activation (Cos et al., 1998). In the case of reducing inflammation, mechanisms such as nitric oxide synthase and cyclooxygenase-2 (COX-2) enzyme induction, as well as other



**Fig. 42.1** (i) Structure of *Opuntia* flavonoids showing possible substitution points (a–d) and 3 major rings (1–3); (ii) A diagrammatic representation of some flavonoid classes (anthocyanidins, isoflavones, flavones, flavonols). (Adapted from Heim et al., 2002 with permission from Elsevier)

inflammatory enzyme and pathway inhibition, have been reported for bioactive plant compounds (Togna et al., 2014). Bioactive plant compounds also cause cancerous cell death by upregulating some pro-apoptotic proteins' expression to bring about their anticancer action. Cancerous cells may also experience a reduction in membrane depolarisation potential and condensation of chromatin shrinkage in response to biologically active compounds (Lin et al., 2014).

### 3.5 Cosmetics

Very few cosmeceutical companies are aware of the utilisation of nopalitos in making shampoos, gels, creams, and lotions. This application is underexplored, possibly because the properties of the *Opuntia* for this use are not widely known, thus limiting demand for them. Again, cosmetics do not necessarily require considerable amounts of plant material during production (Corrales & Flores, 2003). Nopalito powder added to make-up and skincare products reduces melanin and cytokine production and protects against free radical generation. A 14% reduction in pigmentation (melanin production), a 31% decrease in facial wrinkles, decreased UV-induced oxidation, and upregulation of skin defence systems was demonstrated in a recent clinical trial using nopalito powder (Naolys, 2018).

### 3.6 *Natural Additives*

Given the growing global awareness of the attendant risks associated with synthetic additives, demand for natural products and additives is rising as regional laws are withdrawing many synthetics. This is so because anything natural is generally regarded as secure and safe, even though this may not always be the case. Additives are in high demand for cosmetics, pharmaceuticals, and foods and are therefore well used in these industries. Examples include carminic acid and gum additives derived from cladodes (FAO, 2013).

### 3.7 *Construction*

Nopal mucilage is useful in the production of binding agents for use in the construction industry. Mucilage is also being explored on steel for its anti-corrosive features (Hammouch et al., 2004). Anticorrosive properties were reported for a mixture of mucilage with a concrete mix poured around steel strengthening rods (Torres-Acosta et al., 2005). When added to lime, nopal mucilage serves as an adherent for paints (Ramsey, 1999). The organic adhesive produced from the mixture of nopal juice and lime is used to protect and restore historical monuments. Flexible ratio mixtures of nopal mucilage and lime result in dried pastes with varying mechanical properties tailored to different applications (Cárdenas et al., 1997). In a study undertaken in 2004, *Opuntia* mucilage added to cement mixtures improved the cement products (Torres-Acosta et al., 2004). Mucilage has also uses as a stucco water barrier and brick and adobe plaster.

### 3.8 *Tourism*

Mature lignified cladodes have been useful for making artisan crafts such as baskets, bangles, artifacts, and earrings, and find applications in the tourism sector (FAO, 2013).

### 3.9 *Water Clarification and Wastewater Treatment*

Cladodes have been used for water clarification (Table 42.1). *Opuntia stricta* var. *dillenii* and *O. ficus-indica* mucilage have been shown to effectively clarify medium to highly turbid drinking water samples compared to the conventional aluminium

sulphate [ $\text{Al}_2(\text{SO}_4)_3$ ] agent. No disagreeable odour was recorded following the water treatment. The effective reported concentration was 0.8 mL mucilage per litre of water. Also, iron (Fe), aluminium (Al), and manganese (Mn) heavy metals were removed, while faecal coliforms and the water chemical oxygen demand (COD) decreased (López, 2000).

In another study, *Opuntia* cladode mucilage coagulative properties were high in water within the basic pH range (pH 8–10) and up to 98% removal of water turbidity. Dose increased as the initial water turbidity increased under experimental conditions (Miller et al., 2008). Another natural coagulant, *Moringa oleifera*, showed similar coagulative action. The mechanism of action for the coagulative capability of *Opuntia* cladodes varies and remains a subject of research. Nonetheless, a study has pointed to the mechanism being linked to adsorption and bridging or sweep flocculation action. In this mechanism, particles make contact, bind together, and form a polymer material which constitutes the floc. The optimised use of natural coagulants in water treatment provides a cheap and practical option in developing areas (Miller et al., 2008). Wastewater clarification has also been reported for *Opuntia* hydrocolloids by some authors (Domínguez López, 1995; Muñoz de Chávez et al., 1995; Viguera & Portillo, 2001; Anderson, 2001; Sáenz et al., 2004).

### 3.10 Agricultural Uses

The improvement of drainage, organic matter, infiltration, or seeping of water into soils has been reported as an application for nopals (Gardiner et al., 1999). The *Opuntia* is useful as a hedge plant. *Opuntia*-based biogas production plants have delivered steady nutrient-rich wastewater for irrigation and fertilisation of food crops grown close to the gas generation plant (FAO, 2013). A biofertilizer made from nopalitos and animal manure was reported to improve plant biomass by providing soil nutrients (García, 1994), and enhanced sprouting and root development in *Opuntia* plants (García de Cortázar & Nobel, 1992). Compared to fresh farm manure, biofertilizers, including *Opuntia*, showed better stability for biogas production and easier to handle. Biofertilisers also improve the biological, chemical, and physical characteristics of the soil.

### 3.11 Insect Repellent

The *Opuntia* has been traditionally used as an insect repellent, but no publication exists that explicitly demonstrates the technique, use, and scope. According to the FAO (2013), a patented product is available for sale in Honduras.

### **3.12 *Nopal Residues in Vermiculture and Biofertilizer Production***

Vermiculture refers to earthworm cultivation on biodegradable organic materials, which have undergone semi-composting. The resultant material is a worm cast or vermicompost, added to soil as a biofertilizer. One such biofertilizer is made from nopalitos with cattle manure and improved agricultural production systems' sustainability and efficiency, especially in arid areas (García, 1994).

### **3.13 *Drought Survival***

Recurrent drought is a major problem in arid regions, and *Opuntia* cultivation provides a workable solution to farmers in these areas. The nutritive and succulent nature of the crop enables livestock growers to carry on despite harsh and extended drought periods.

### **3.14 *Other Non-Food Opuntia Cladode Applications***

As far back as the late 1920s, cladode spines had been patented for use as gramophone needles (Ramsay, 1928). Fuel combustion had also later been enhanced with the addition of *Opuntia* cladode extract (Scifoni, 1985). *Opuntia* stem hydrocolloids (pectin and mucilage) may also be applied to produce new biomaterials and bioplastics for biomedicine and packaging materials production (López-Palacios et al., 2016; Medina-Torres et al., 2000).

## **4 *Opuntia* Fruit Applications**

The *Opuntia* fruits show variable skin thickness, low acidity, vary in colour and are pulpy and juicy with good sugar content. Once harvested, *Opuntia* fruits do not ripen; that is, they are non-climacteric. Thus, fruit harvest indices should be based on each type of fruit in a specific harvesting area. It has been reported that the best harvest time for *Opuntia* fruits could be determined based on indices such as fruit size and fill, fruit firmness, flower receptacle depth, fall of the glochids, change in fruit peel colour, and content of total soluble solids (TSS) (Inglese, 1999; Cantwell, 1999).

*Opuntia* species can be differentiated by the characteristics of the fruits they produce. *O. streptacantha* fruits are juicy and sweet but purple. *Opuntia ficus-indica* fruits are sweet and colour varieties range from red to purple to orange and yellow.

The *Opuntia xocconostle* fruits are more acidic, pink, or purplish-green on the insides and smaller compared to the *O. ficus-indica* fruits (Scheinvar, 1999). In terms of purposeful cultivation, Mexico has the largest expanse of land under *Opuntia* fruit production. Other countries with dedicated areas for *Opuntia* fruit production include Italy, Chile, Peru, Spain, South Africa, North African countries (Egypt, Tunisia, Morocco, and Algeria), Venezuela, Argentina, Bolivia, Jordan, Israel, and the United States (Barbera, 1999). In these countries where awareness of the *Opuntia* plant's potential benefits exists, the fruits are used as raw materials in the food, cosmeceutical, and pharmaceutical industries. The fruit chemical composition, ripening stage, pulp yield, and property of the desired industrial end product must be considered before choosing the processing conditions and techniques to derive high quality and safe products. The *Opuntia* plant growth location and prevailing climate have been reported to impact the plant parts' mineral and phytochemical profile, causing variations in fruit content (FAO, 2013). Several *Opuntia* fruit applications are outlined and discussed in the following paragraphs.

#### 4.1 Food, Beverages, and Other Food Products

Vegetables and fruits are industrially processed into beverages and a wide array of products in order to achieve;

1. Employment generation,
2. Improve shelf life and quality before reaching the target market,
3. Product price stabilisation, especially in the case of excess market supply,
4. Product availability all year round, and
5. Value-added finished products with better marketing potential.

On the offside, the *Opuntia* fruits' large scale use and marketability are considerably reduced because the fruits spoil rapidly and are fragile. Their use is mainly directed towards producing various fermented beverages and foodstuffs (López et al., 1997). *Opuntia* fruits have also been processed and preserved into syrups, which can last for long periods without spoilage. When the fruit pulp is boiled, a toffee product named 'melcocha' is produced, and on further processing results in an *Opuntia* fruit-based cheese (Table 42.1). When sun-dried, the fruits have also yielded cactus pear raisins or dried fruits (Corrales & Flores, 2003). Other food products prepared from *Opuntia* fruit processing include nectars, gels, juices, jams, liquors, and juice concentrates (FAO, 2013). Sweeteners, vinegars, wines, canned fruits, frozen pulp, and fruit products are also possible (Sáenz, 2000; Corrales & Flores, 2003).

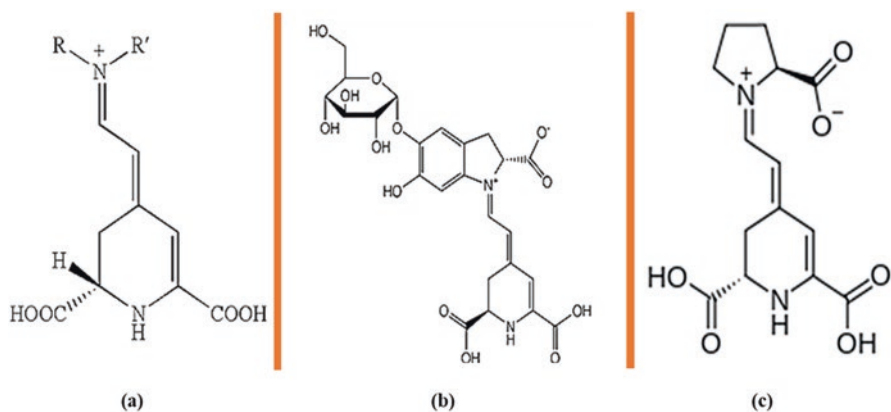
Colonche made from *Opuntia streptacantha* juice is a low-alcohol fermentation product produced in Mexico. Its production involves the use of *Saccharomyces* sp. as a starter culture. Aguardiente (brandy) and wine (Table 42.1) are *Opuntia*-based products made by *O. streptacantha* and *O. robusta* (Corrales & Flores, 2003). However, the fermentation and production processes can still be modified to

produce different beverages and liquor brands. This is also the case with vinegar production.

The *Opuntia* nopal and fruits are rich in functional compounds which form the basis of most new food formulations and functional foods. Examples of some functional components are fibre, hydrocolloids, carotenoid, and betalain pigments, potassium and calcium minerals, ascorbic acid (vitamin C) (Sáenz, 2004; Díaz et al., 2017), and an array of phytochemical antioxidant molecules (Aruwa et al., 2019b) including macromolecular antioxidants (MAs) (Aruwa et al., 2019a; Serra et al., 2013). Functional components contribute to a healthy diet and are also integral to designing new food formulations and functional food. Functional food formulations that incorporate one or more functional ingredients are targeted towards providing physiological benefits such as disease therapy or prevention, improvement of mental and physical well-being, and health enhancement (Sloan, 2000).

## 4.2 Pigment Production

Betalains (Fig. 42.2a-c) are biologically active phenolic compounds and pigments found in *Opuntia* fruits. Betalains consist of two major compound classes; betacyanin (i.e., betanin Fig. 42.2b), and betaxanthin (i.e., indicaxanthin Fig. 42.2c). Betalain pigment derivation and extraction from purple and red *Opuntia* fruits are possible. Other colour varieties remain unexplored, even though the orange *Opuntia* fruit has relatively good levels of carotenoids. These pigments have applications in the food industry as colourants and additives (FAO, 2013).



**Fig. 42.2** (a) The structure of betalain pigments (b) structure of betanin, a common betacyanin compound with antioxidative potential, and (c) structure of a betaxanthin compound, indicaxanthin. (Adapted from Khan & Giridhar, 2015 with permission from Elsevier)

### 4.3 *Pharmaceutical Uses*

Due to phenolic acids, betalains, flavonoids, and other phenolic antioxidants in *Opuntia* fruits, some pharmacological properties have been linked to *Opuntia* fruit extracts. Some of these include hepatoprotective, cardioprotective, antidiabetic, neuroprotective, anti-inflammatory activities (Serra et al., 2013), and antioxidative activity (Albano et al., 2015; Alimi et al., 2013). Anticancer properties have also been recorded (cervical cancer at an effective concentration of 100µg/mL extract) linked to fruit extract content of dihydrokaempferol, *trans* taxifolin, and flavonoids (Hahm et al., 2015). Hepatic, colonic, and prostate cancer cells have also been inhibited by *Opuntia robusta*, *O. violaceae*, and *O. rastrera* fruit extract (Chavez-Santoscoy et al., 2009). *Opuntia ficus-indica* varieties cultivated on drainage sediments showed increased selenium levels and chemotherapeutic activities (Bañuelos et al., 2012). A new (1→4)- $\alpha$ -D-glucan polysaccharide compound was identified in *O. ficus-indica* fruit fractions (Ishurd et al., 2010). Both known and new compounds identified and derived from *Opuntia* fruits could be harnessed to provide health benefits. Of the many components of the *Opuntia*, fruit fibres are the most studied, and their link to health and wellness are well understood, such as the prevention of obesity, diabetes, and control of cholesterol (Hollingsworth, 1996; FAO, 2013).

### 4.4 *Fruit By-Product Applications*

Production efficiency is significantly increased when agricultural industries optimise raw materials received into the production area. This is where *Opuntia* by-products like *Opuntia* fruit seeds and peels come in. The processing of by-products into commercial value products reduces waste, boosts company income, and reduces enterprise costs (FAO, 2013).

Before processing fruit juice into various products, fruits are deseeded, and the seed is set aside or discarded. However, the fruit seed by-products may be useful in deriving specific products such as edible oils. The seeds contain oils that are edible and rich in linoleic and other unsaturated fatty acids. Oil yields can reach up to 17%, which favourably compares with known oilseeds for possible commercial exploitation (El Kossori et al., 1998). The optimal use of plant by-products contributes to their valorisation and waste materials management. The seeds may also be used as components in animal feed (Sawaya et al., 1983). Sulphur, amino acids, and minerals are richly present in *Opuntia* seeds (Sawaya et al., 1983). Other potential *Opuntia* seed chemicals for use in various industries include vitamins from *O. macrorrhiza*, organic acids, and sugars (Chahdoura et al., 2015), sterols from *O. ficus-indica* *O. dillenii* (Ghazi et al., 2013), and an array of phenolic compounds groups (flavonoids and tannins) (Chougui et al., 2013). *Opuntia* seed oils have demonstrated anti-corrosion capability (Hmamou et al., 2012), and the reduction of animal feed conversion efficiency when used as a feed supplement (Ennouri et al., 2006).



#### 4.5 *Other Opuntia Fruit Products*

An equivalent of cocoa butter (Table 42.1) was produced from the growth of *Cryptococcus curvatus* on *Opuntia* fruit juice. Cocoa butter is useful in producing chocolate, toiletries, pharmaceuticals, and ointments (Jana, 2012).

### 5 *Opuntia Peel Applications*

The amount of *Opuntia* peel by-product derived from *Opuntia* fruits varies depending on the plant's cultivation location and growth stage. The thick *Opuntia* peel is rich in mucilage, high in fibre, and protects the fruit against excess water loss (Sepúlveda & Sáenz, 1990). Some *Opuntia* peel and peel powder applications are discussed below.

#### 5.1 *Food Products*

Peels from *O. xocconostle* have been used in sauces made in the United States and Mexico (Sáenz, 1999). A considerable amount of hydrocolloid mucilage is extractable from *Opuntia* peels for utilisation as a thickening agent. The high fibre content of peels makes them suitable raw materials for prebiotic preparations and antioxidants. They may also be subjected to fermentation using cultures such as bifidobacterial and lactic acid bacteria (LAB) to generate new compounds for expanded applications (Diaz-Vela et al., 2013). Peel powders rich in antioxidants used to supplement wheat flour enhanced the nutritional properties of baked bread, while decreasing staling and increasing bread shelf life. Peels and peel powders are also used as water and oil retention capacity improvers and substitutes for sugar and fat to stabilize oxidative processes in food (Anwar & Sallam, 2016). *Opuntia* peel fractions have been added to margarines and showed improved oxidation resistance compared to vitamin E-supplemented margarines. The extract did not negatively impact the margarine's microbiological and physicochemical properties (Aruwa et al., 2018; Chougui et al., 2015).

#### 5.2 *Betalains and Other Pigment Production*

As is the case with the *Opuntia* fruits, the purple and red *Opuntia* cultivars are useful in deriving natural betalain pigments (Table 42.1 and Fig. 42.2a–c). Betalain (betanin) (Abou-Ellella & Ali, 2014) and other pigments like hydrocarbon carotenes, xanthophylls, and chlorophyll (Cano et al., 2017) have been identified in *Opuntia*

peels. The production of betalains and other pigments from *Opuntia* peels needs to be optimised for commercialisation.

### 5.3 Pharmaceutical and Medical Uses

Dried peels from *Opuntia ficus-indica* serve as dietary supplements and functional food (Fig. 42.3) in places like the United States. This use is linked to its ability to reduce the hangover effect associated with excess alcohol consumption (Wiese et al., 2004). Thus, new potential applications continue to emerge from research on the *Opuntia* species, even in the medical industry. Carbohydrates, fatty acids, antioxidants, vitamins (Cerezal & Duarte, 2005), and lipids (Ramadan & Mörsel, 2003) are derived from *Opuntia* peels. They could be channelled toward producing pharmaceuticals that provide specific health benefits such as antimicrobial, antioxidant, and anticancer benefits (Fig. 42.3). Peel extracts used to supplement animal diets influenced the reduction in cholesterol levels *in vivo* (Milán-Noris et al., 2016). Peel

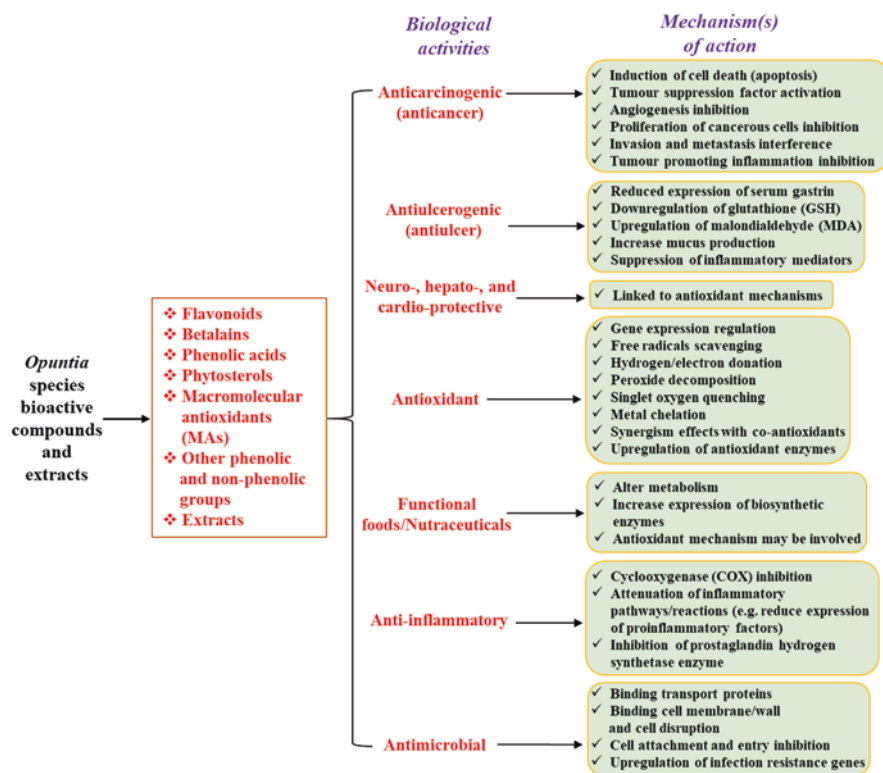


Fig. 42.3 An overview of *Opuntia* compounds and extracts, biological activities, and action mechanisms

extracts have also been used against hypertension (Federici et al., 2009), and cancer (Abou-Elella & Ali, 2014).

Plant sterols (Fig. 42.3) such as those derived from *Opuntia* species, decrease lipid absorption (Aruwa et al., 2018; Bartnikowska, 2009). Phytosterols may raise antioxidative enzymes' levels, thus bringing about cell death (Soodabeh et al., 2014). Within living organisms, *Opuntia* peel extracts have shown hypocholesterolemic action linked to extract's ability to block bile acid transportation and cholesterol absorption and inhibit liver HMG-CoA enzyme activity (Milán-Noris et al., 2016). *O. ficus-indica* peel extracts have also demonstrated the ability to increase the amount of calcium in cancer cell lines (Jurkat T-cell), and cause the cancerous cell membrane to be hyperpolarised (Aires et al., 2004).

Generally, the extent to which biologically active plant compounds are branched, their composition, structure, absence, or presence of sugar molecules, and their physicochemical attributes go a long way to determine such compound's pharmacological activity profile (Koubaa et al., 2015). Most bioactive phytochemicals are aromatic. Flavonoids and other phenol classes are hydroxylated aromatics (Ciocan & Bara, 2007). The number of hydroxyl groups present in the structure of a compound impact its activity range. A high number of hydroxyl groups favours antimicrobial activity in a compound. Antimicrobial action occurs through the compound's ability to destroy the membrane integrity of microbial cells and cultures (Ciocan & Bara, 2007). For example, quinone molecules bind to extracellular proteins and penetrate the cell membrane to cause a killing or static effect. Phenolic coumarin compounds act *via* a similar mechanism. Polymeric phenols with multiple numbers of hydroxyl groups generally show enhanced antimicrobial action. Examples include tannins, which may be in the condensed or hydrolysable form. Polymeric tannins inactivate microbial enzymes and adhesins, bind cell walls, and transport proteins, thus inhibiting protease activity and preventing microbial growth (Ciocan & Bara, 2007).

#### 5.4 Other Peel Products

Citric acid was economically produced through solid-state fermentation of *Opuntia* peels using *Aspergillus niger* (Jana, 2012) and could serve various industries. Hydrocolloids from *Opuntia* peels may also find added value in their use in making new thickening and film producing agents and emulsifiers in cosmetics, foods, and pharmaceuticals. Biomaterials production (Table 42.1) for biomedicine and packing materials are also possible (Peña-Valdivia et al., 2012; Rodríguez-González et al., 2014). *Opuntia* peels as plant by-products have also been channelled for biogas (Gebrekidan et al., 2014), and biofertiliser (Quintanar-Orozco et al., 2018) production. Production of products such as bioprotein from *Opuntia* peels have also been reported and have application in the chemical and pharmaceutical industries as additives (Gad et al., 2010). Future research and development (R&D) studies on *Opuntia* peel potentials would not only provide greater insights and knowledge but broaden knowledge on their application(s) for many industries.

## 6 Factors Affecting the Commercialisation of *Opuntia*-Based Products

Some of the factors that impact and influence the successful or unsuccessful commercialisation of *Opuntia*-based products are highlighted and discussed in this section.

### 6.1 *Opuntia* Fruit Softness and pH

These characteristics (Fig. 42.4) make *Opuntia* fruit processing a peculiarly arduous task. The softness of the fruits, when ripe, make them prone to rapid spoilage. Rapid spoilage then leads to agricultural losses and reduced economic and financial gains to farmers. This could be circumvented by using specialised storage facilities and



Fig. 42.4 Factors affecting the commercialisation of *Opuntia*-based products

fresh fruits or processing fruits into various forms that would aid their preservation before consumption, use, or sale. Fruits may also be harvested some days before they become fully mature. Maturity is dependent on the harvester's need and intended use for the fruits. Again, *Opuntia* fruits are within the acidic pH range of 5.3 to 7.1, low acid group with a pH > 4.5. However, a pH of  $\leq 3.5$  has been reported for *Opuntia xocconostle* fruits (Mayorga et al., 1990). Heat treatment of the fruits is, therefore, necessary to achieve microbial growth control. This treatment is usually done at a temperature minimum of 115.5 °C and is important since the pH range value of the fruits and their high TSS creates a favourable environment for the growth of spoilage microorganisms.

## 6.2 *Opuntia Nopal and Fruit Seasonality and Perishability*

*Opuntia* fruits are perishable and seasonal. The high water content of nopalitos makes them perishable, but nopals are not seasonal. Therefore, effective preservation is necessary to avoid all forms of spoilage linked to physical, biological, nutritional, chemical, or enzymatic changes. Preservation also ensures their availability as raw materials for various industrial processes for most of the year. Preservation techniques need to be carefully selected to prevent tremendous losses. Biochemical (alcoholic or lactic fermentation), chemical (preservatives, sugar addition, acidification, and salting), and physical (dehydration, canning, refrigeration, pasteurisation, freezing, electric pulsing, high pressure, and irradiation) preservation methods are some of the effective techniques being used. One or more preservation methods can be combined to achieve the best possible result. Further studies are still required to determine the best preservation and processing method(s) to be used since these differ for *Opuntia* species and depend on several other factors.

## 6.3 *Pulp Viscosity*

The presence of hydrocolloids such as mucilage and pectin make *Opuntia* fruit pulp highly viscous. These hydrocolloids are important components of the *Opuntia* dietary fibres. Pulp viscosity can, however, be harnessed and developed into food thickeners upon extraction of the hydrocolloids from the pulp (Sáenz et al., 2004). These hydrocolloids may favourably compete with existing thickening agents such as guar, locust bean gum, and other gum types (Goycoolea et al., 2000; Medina-Torres et al., 2003).

#### **6.4 Processing Pitfalls and Harvesting Peculiarities**

Fruit aroma has been reported to change during processing. A sweet aroma turns to one which smells like grass or hay during heat processing to produce certain products. This factor is a challenge that future studies could aim to solve. However, change in the aroma is mainly dependent on the processing need for the desired product. Another recorded pitfall is the darkening of cladodes and nopals due to phenolics, which make preservation difficult (Rodríguez-Félix, 2002). Also, during harvesting, the nopals' acidity needs to be considered because young cladodes' acidity levels vary during the day due to CAM. Preservation of the stems can be facilitated using their acidity, and the acidity can also improve consumer acceptability of the end product. Processing and storage technologies after harvesting could also be improved to reduce losses.

#### **6.5 Farm Management and Pest Control**

A good example under this challenge is with cochineal breeding for grana or carmine production. It is advised that agricultural space for cochineal production be done separately from other nopal and fruit planting areas. Such demarcation and defining of boundary lines are practiced in the Canary Islands and Chile, where cochineal is commercially produced (FAO, 2013). This avoids problems with pests on the nopal and fruit farms. Prevention and control of insect pest attacks on *Opuntia* plantations is an integral crop management activity.

#### **6.6 Restriction to Use as a Weed and Low Awareness Level**

Many countries and regions to which *Opuntia* plants have spread still view the plant as a weed and nuisance which takes up useful land space. As a result, they are cut and burned off. This is, however, linked to a lack of knowledge (Fig. 42.4) on the potential applications of the plant. While some regions where the *Opuntia* is indigenous have explored the plant's benefits over time, many countries still lack awareness of the *Opuntia* species capacity for subsistent and commercial gains to individuals, groups, and nations. Awareness may be broadened using all possible and available media such as the television, radio, and social media platforms. Such platforms could be dedicated to educating the public, companies, potential investors, governments, and industries on the potential socio-economic and financial gains from the *Opuntia* plant's growth and use.

## 6.7 *Lack of Enabling Policies*

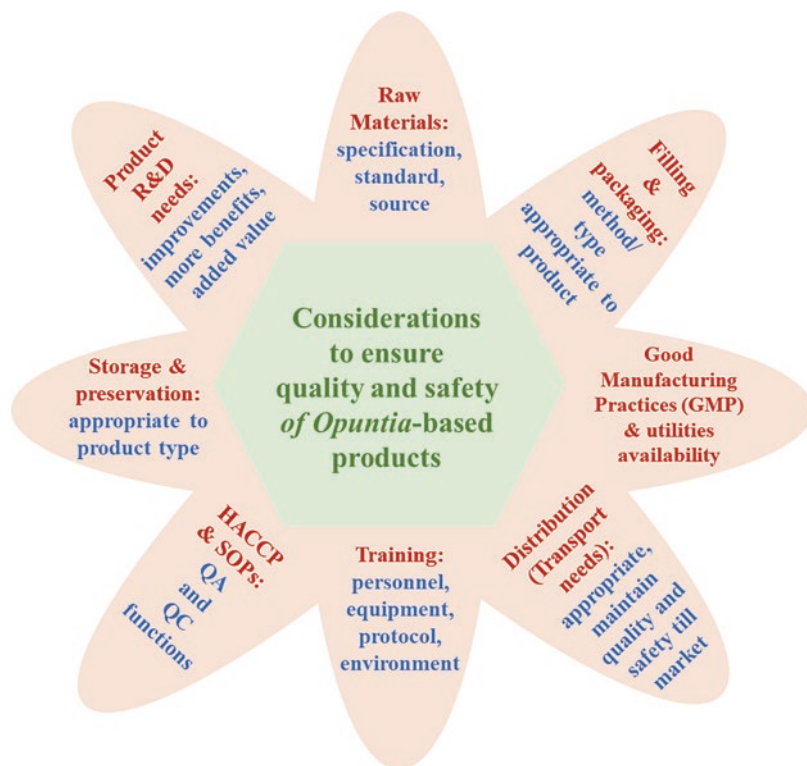
National and international policies to aid the acquisition or use of land for large scale agricultural cultivation of the *Opuntia* plant remain a major challenge in many countries. This is more prominent in low and middle-income developing countries. Bureaucratic bottlenecks within the agricultural sector bars discourage herders and farmers who could want to venture into *Opuntia* cultivation. To tackle this, governmental agencies need to be sensitised regularly on the potential profits and economic gains derived from the cultivation of *Opuntia* species for food and specific industrial applications. Associations such as the Cactus Network (CactusNet), National Association of Cactus Development (ANADEC), and Cactus and Succulent Specialist Group (CSSG) need to expand and open new branches worldwide, especially in developing regions where there are no such associations. These may be able to favourably impact policies that could aid in facilitating the cultivation of *Opuntia* species. Regular papers, bulletins or publications from cactus associations and scientific researchers in cactus pear-focused journals [Journal of the Professional Association for Cactus Development (JPACD) and Cactus and Succulent Journal (CSJ)] on emerging and trending topics on the *Opuntia* plant and their applications can be integral to keeping governments informed, updated, and also impact policies.

## 6.8 *Research Needs*

While research on the *Opuntia* species is on the rise, many related species and variants still require in-depth research to expand our knowledge on potential new applications and benefits. *Opuntia* applications may be broadened beyond what is already known. New knowledge and research data continue to emerge on the plant, especially from different geographical locations, as the phytochemical profile of similar *Opuntia* species show major variations. The exploration and exploitation of the array of known and new compounds derivable from *Opuntia* species remain largely understudied. Future agricultural, scientific, and multidisciplinary research could contribute immensely to bridging the research and knowledge gap around the *Opuntia* species.

## 6.9 *Quality and Safety Concerns*

Quality and safety (Figs. 42.4 and 42.5) are measured mainly by adherence to set acceptable standards for various product categories irrespective of the industry they serve. Since most *Opuntia* products are largely distributed in regions aware of the plant's potential, attaining international quality standards may be an arduous task. Nonetheless, current national and international food quality standards that apply to



**Fig. 42.5** Quality and safety parameters to be considered in *Opuntia*-based product manufacturing. *HACCP* Hazard Analysis and Critical Control Point, *SOPs* Standard Operating Procedures, *QA* Quality Assurance, *QC* Quality Control, *R&D* Research and Development

respective food categories can be used while *Opuntia* product awareness gradually reaches international acclaim and pending the development of quality standards, especially for new product formulations.

Setting standards facilitates collaboration along the value chain. The use of standard operating procedures (SOPs) (Fig. 42.5) within production industries goes a long way to improve consumer protection. A Codex Alimentarius standard exists for prepared, packed, and marketed cladodes, that is, the CODEX STAN 185–1993. No international standard exists for brined and pickled nopal products which are processed industrially (FAO, 2013). Brands, seals, and certification of products that eventually reach the target market are dependent on compliance checks. All stakeholders and local, national, and international inspectorates and regulatory agencies have a crucial role in ensuring and maintaining compliance with set standards (FAO, 2003).



Hygienic practices go a long way to ensure and assure product safety. The role played by good manufacturing practices (GMP) (Fig. 42.5), and implementation of hazard analysis and critical control point (HACCP) principles cannot be overemphasised in achieving food safety and quality. Maintenance of quality and safety from farm to fork is likewise an imperative, as they impact the quality of the finished products. This is where good quality control and assurance activities play essential functions.

### **6.10 Marketability**

Potential investors must develop experience with market exploitation and identification. Substantial market research (Fig. 42.4) before the start-up of *Opuntia*-based processing or production firm on any scale, whether small, medium, or large, is essential. In some instances, insufficient market capacity may exist. Product brands and earned seal or certification, safety, quality, labeling, and presentation tremendously impact on *Opuntia* products' price and marketability (Fellows, 1997). Given the increased consumer awareness, some, if not all consumer groups, tend to appreciate the inclusion of a product's raw material origin on the product labels, provided they are truthful. These qualities set products apart, especially from similar ones in the market, even those made from other fruits and vegetables. Novelty backed by consumer acceptance could also boost *Opuntia*-based products' marketability. Trial-runs on a small production scale with small product samples could give a pointer to the potential prospect. Product differentiation based on functional and nutritional (improved fibre, antioxidant and mineral content), and physical (aroma, taste, texture, appearance) product characteristics could also be key to the successful sale and acceptance of a product (Fig. 42.4). Unique marketing campaigns, such as those highlighting high-value products and novel products' functionalities, could suffice to improve *Opuntia*-based products' marketability.

### **6.11 Filling, Packaging, and Storage Peculiarities**

Producers of *Opuntia*-based products must take time to familiarise themselves with their product(s) of choice before processing and production. Finished product filling, packaging, and storage before, during, and after production has to be carefully thought out and selected to suit the end product characteristics; otherwise, the entire production process can experience immense wastage if care is not taken (FAO, 2013).

### **6.12 Economies of Scale**

These have to do with weighing business capital for successful operations, as well as infrastructure needs and location, equipment, transportation, target market proximity, utilities, and services (water, electricity, energy), and raw material, and labour availability considerations, among others. Training and cleaning routines should also be factored in. A business plan should be drawn, backed by a feasibility study that addresses these economic aspects. This is integral to reducing the risk of business failure and deciding if a venture is worth starting or investing in.

### **6.13 Land and Credit Access**

Access to land and credit facilities is a limitation in the agro-industrial utilisation of *Opuntia* species. Terms and conditions attached to accessing credit and loan facilities and letting and purchasing arable land for cultivation or production processes vary from region to region. The terms could be continuously evaluated to make land and credits more accessible to subsistent, medium, and large scale investors and growers to boost *Opuntia* species' cultivation for food and non-food applications (FAO, 2013).

### **6.14 Limited Extension Services and Technical Know-How**

Farmer, investors, and stakeholders need to garner efforts to create extension services (Fig. 42.4). Increasing and improving extension services could help bridge the gap of ignorance and increase technical knowledge concerning *Opuntia* cultivation and applications after harvesting. These are integral to getting interested persons, organisations and industries to fully grasp, understand, manage, control and exploit the many opportunities open to them through *Opuntia* cultivation and application. Cactus pear associations may also provide technical assistance where and when required. Training on modern techniques used in other countries may also boost the technical capacity of newcomers and experts.

### **6.15 The Presence of Spines on Some *Opuntia* Varieties**

Spiny *Opuntia* species need regular supervision and pose a major problem. The spines are useful when the plant is used as a hedge or living fence. Innovation is still required to define, design, and evaluate techniques that may be useful for integrating the spiny variants.

In summary of this chapter, the *Opuntia* plant's industrial applications need to be integrated across several industries. Integration is possible when demand and supply and the efficient utilisation of raw materials can be managed and optimised for greater profits and net returns to stakeholders and investors. In regions tackling food security issues, the populace's empowerment through encouraging *Opuntia* species cultivation and *Opuntia*-based products manufacturing can help reduce food insecurity. This is possible since the *Opuntia* plant can grow where others cannot. *Opuntia* species have multiple advantages, which are evident in their many uses in the agro- and other industries compared to other plant species. Also, many non-food applications are itemised here, which are of special value and interest to many industries. These non-food uses require exploitation on an industrial scale. Good manufacturing practices and hygiene must not be overlooked in industrially valuable products from the *Opuntia* plant. A lot still needs to be done concerning setting acceptable standards and thresholds across relevant industries to ensure uniformity in *Opuntia* products that eventually reach the market. The *Opuntia* plant is a perennial which could be exploited as a naturalised resource, even in the wild. Such exploitation should be expanded to include new species and cultivars. The introduction of new management practices, methods, and institutional capacity within the *Opuntia* value chain would bolster the industrialisation and use of the *Opuntia* plant globally.

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# Chapter 43

## *Opuntia* spp. as a Source of Sugars for the Ethanol Production



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**Abstract** *Opuntia* spp. is a plant with great potential for developing biotechnological processes. Its rapid growth and adaptability to dry and semi-dry climates make the plant an excellent candidate to implement biotechnological processes to obtain by-products. *Opuntia* spp. is capable of storing large amounts of water in its cladodes which is the main biopolymer responsible for this is mucilage. This biopolymer comprises various sugars such as xylose, arabinose, and fructose. They have great potential to be used as raw material to formulate production and microbial growth media. *Opuntia* cladodes contain, according to their age, different concentrations of lignin and cellulose. The concentration of cellulose within cladodes increases depending on the age of the plant and this feature can be used to increase glucose concentration within the *Opuntia* hydrolyzates and improve the yields of by-products such as bioethanol. A highly important factor in the availability of monosaccharides within *Opuntia* hydrolyzates is the hydrolysis method. A great diversity of these methods has been described. However, the sugar yields are only increased when physical, chemical, and biological methods are used in combination. Using *Opuntia* spp. as raw material for obtaining sugars has several advantages, among them: its availability throughout the year, the low cost of cladodes and the low demand for plant care, making *Opuntia* excellent raw material for the implementation of biotechnological processes.

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## 1 *Opuntia* spp. Overview

The genus *Opuntia* comprises 377 species; of these, about 48 species are used by man, 24 are used for direct consumption, 6 species are used for its fruit, 15 species such as forage, and three species such as vegetable. Of all recognized species, the most common and cultivated in different parts of the world is *Opuntia ficus-indica* (Ornelas, 2011). This species is a member of the cacti that is adapted to semi-arid regions because they have a crassulaceous acid metabolism (CAM) through which the stomata are closed during the day and open at night, avoiding the loss of water through respiration (INEGI, 2013). It contains low levels of dry matter, crude protein and fiber, and high levels of soluble carbohydrates (Habibi et al., 2009; Tosto et al., 2015).

### 1.1 *Opuntia* Productivity

Vegetable productivity can be measured in various ways, such as the number of fruit boxes harvested, fiber weight, grain yield, or total air-dry weight produced (Gallegos & Méndez, 2000). Productivity itself reflects the cumulative effects of many factors on growth. In natural environments, factors such as type of soil, availability of water in the soil, temperature, photosynthetic photon flux (FFF), and the nutrient content in the soil determine the establishment, development, growth, and production of the plants (Nobel, 1994).

The knowledge of the potential of using the agricultural lands is a powerful tool for the achievement of less vulnerable agriculture with a greater probability of success. The selection of species with agroecological potential for a region implies advantages in crop management. Producing a species outside its optimal environment makes production technologies more expensive (Chang, 1981). Each plant species has specific environmental needs for its growth and development. These needs or requirements are normally described by rank and usually reported by species (Benacchio, 1982; FAO, 1993).

*Opuntia* is a plant that responds favorably to management, mainly to the application of water and fertilizers. Thus, in nopal cultivation, when attention is paid to the adequate spacing and other management practices (moisture availability, optimization of soil fertility, thermal index, etc.), the annual productivity of *Opuntia* can be extraordinarily high (Gallegos & Méndez, 2000). Barbera (1995) indicated that under the best environmental and cultural conditions, productions of up to 35 t of fruit ha<sup>-1</sup> year<sup>-1</sup> have been obtained in southern Italy. A similar yields to those

reported by Gallegos et al. (2004) for the Juchipila Canyon region, for Apastillada, C-17, Cristalina, and Burróna cultivars with 35.8, 35.5, 34.9, 33.8 t ha<sup>-1</sup> respectively. Bravo and Medina (2003) reported that applying two irrigations yields up to 25 t ha<sup>-1</sup> prickly pear.

## 1.2 *Opuntia* Uses

There are many products obtained from the cactus fruit, some of them are known, and others have been recently developed or are in the process of research. Regarding the products obtained from cladodes, many research works are directed to producing food and other by-products (Table 43.1). Among many applications of *Opuntia*, some are used as a host for the cochineal insect (*Dactylopius coccus*), the nopalitos (cladodes) are also used to make antidiabetic preparations, their flowers are used to prepare diuretic drinks, and the fruits are used to prepare juices, jellies, honey, jams and pasta, as well as the oil is extracted from its seeds (Reyes-Agüero et al., 2006). As a functional food, fruits and cladodes are an important source of fiber, hydrocolloids (mucilages), pigments (betalains and carotenoids), Ca and K, and vitamin C (Valencia-Sandoval et al., 2010).

## 2 *Opuntia* Composition

According to Saenz (2000), *Opuntia* has good productivity. With 5 years of age, one hectare can produce up to 100 tons of fresh cladodes per year in areas with 150 mm of annual rainfall. The *Opuntia*'s chemical composition is highly variable depending on the species, age of the cladodes, and time of year, with the values generally higher in winter than in summer and the fluctuations higher for *Opuntia ficus indica* compared to *Opuntia amyclae* (Abidi et al., 2009). In cladodes less than 1-year-old, the concentration of total carbohydrates varies in a growing cycle from lower to higher between April and October, a period in which the harvest occurs and the growth of the cladodes stops (Martínez-González et al., 2001). *Opuntia* species

**Table 43.1** Products and by-products of *Opuntia* fruit and cladodes (Saenz, 2000)

Product		By-product
Fruit	Cladodes	Fruit Cladodes
Juices and nectars	Pickles and brines	Seed oil
Jams and Jellies	Sweets	Cladodes mucilage
Sweeteners	Jams	Shell pigments
Wine	Flours	Dietary fibers
Canned fruits	Alcohol	
Frozen fruits	Sauces	

presents high levels of water in its composition with 91.8%; following in percentage carbohydrates with 5.5% and ashes with 1.58% (Ornelas, 2011). These characteristics make *Opuntia* important crop in arid regions, since it meets a large part of the water requirements of the animals, mainly in the dry period (Torres-Sales, 2010).

Among the main components of *Opuntia* is the mucilage (Table 43.2). This hydrocolloid is a polysaccharide whose molecular weight is approximately  $13 \times 10^6$  g/mol and composed of polysaccharides similar to pectins. The sugar content in these polysaccharides is found in approximate proportions of 47% L-arabinose, 18% D-galactose, 7% L-rhamnose, and 23% D-xylose as the main sugar in addition to 5% of D-galacturonic acid (Cárdenas et al., 1997). The *Opuntia* mucilage's primary structure suggests a linear chain with repeated chains of (1–4)- $\beta$ -D-galacturonic acid bonds and  $\alpha$ -(1–2)-L-rhamnose bonds with trisaccharide side chains. The presence of lateral residues to the main galactose chain presents branches of a complex composition containing L-arabinose residues with links (1–5) to residues of D-xylose oligosaccharide groups (Cárdenas et al., 1997).

In addition to mucilage, *Opuntia* is a natural source of fiber composed of a bilateral network of lignocellulosic residues that hardens with age and provides it with a rigid constitution, increasing thickness and intensifying color (Aquino et al., 2012) (Table 43.3). The moisture content of the fibers can be 8%, with an ash content of 4.03% and a lignin and cellulose content of 33.5% and 18.7%, respectively (Aquino et al., 2012).

Various authors have studied the carbohydrate content as the main constituent of *Opuntia* cladodes, finding neutral sugars such as D-galactose and L-arabinose and others such as L-rhamnose and D-galacturonic acid (Amin et al., 1970; Malainine et al., 2003). Specifically for mucilage, it has been reported to consist of a backbone of  $\beta$  (1  $\rightarrow$  3)-galactose units attached to carbon branches that, in turn, contain D-galacturonic acid, D-galactose, D-xylose, L-rhamnose, and L-arabinose units (Ribeiro et al., 2010).

Regarding the mucilaginous fraction, in a study carried out by Guevara-Figueroa et al. (2010) to determine the content of phenolic compounds in lyophilisates of ten varieties of *Opuntia*, it was found that the phenolics present in the samples was from 2 mg/g to 20 mg/g. Additionally, Santos-Zea et al. (2011) observed concentrations of phenolic compounds from 0.3 to 0.9 mg/g under drying conditions. Chemically it has a complex structure with a certain resemblance to pectin, which is why it is

**Table 43.2** Average constitution of *Opuntia* spp. (Feugang et al., 2006)

Constituent	Cladode dry weight basis	Fresh weight basis	
		Cladode (g/100 g)	Fruit (%)
Water	–	88–95	84–90
Carbohydrates	64–71	3–7	12–17
Ash	19–23	1–2	0.3–1
Fibre	18	1–2	0.02–3.15
Protein	4–10	0.5–1	0.21–1.6
Lipids	1–4	0.2	0.09–0.7

**Table 43.3** Composition of *O. ficus-indica* and comparison with some conventional sources of lignocellulosic biomass for the ethanol production

Composition (% dry weight)	<i>O. ficus indica</i> <sup>a</sup>	<i>O. ficus indica</i> <sup>b</sup>	Sugarcane bagasse <sup>c</sup>	Corn stover <sup>d</sup>	Barley straw <sup>e</sup>
Glucan	23.1	15.3	36.74	37.4	37.1
Xylan	3.9	1.9	20.3	21.1	21.3
Arabinan	3.8	4.0	1.8	2.9	3.8
Galactan	6.4	3.4	0.65	2.0	1.2
Mannan	Traces	1.4	–	1.6	–
Total sugars	42	26	59.49	65.0	63.4
Fermentable sugars	34.3	19.7	57	41	38.3
Lignin	7.9	16	20.57	18.0	19.2
Ash	16.8	–	6.53	5.2	8.2
Protein	7.5	6.42	1.13	3.1	–

<sup>a</sup>Kuloyo (2012). Note: uses a South African cultivar

<sup>b</sup>Ginestra et al. (2009). Note: the author uses a mixture of three cultivars from Italy

<sup>c</sup>Szczerbowski et al. (2014)

<sup>d</sup>Ruth and Thomas (2003)

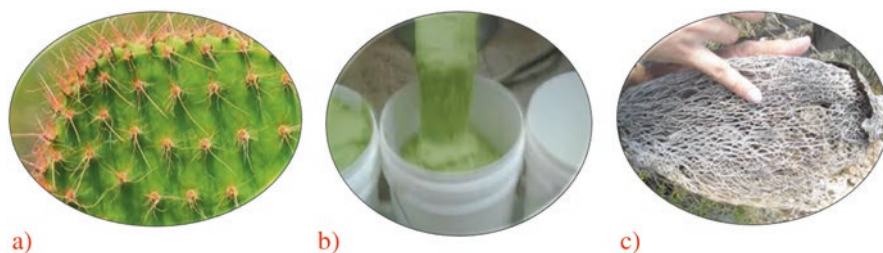
<sup>e</sup>García-Aparicio et al. (2011)

part of dietary fiber. The mucilage properties give it a varied potential for application in food, one of them being a fat imitator and a carrier of flavors. Its ability to stabilize foams is also mentioned by Sáenz et al. (2004).

Another important option is the integral use of the fruit pulp outside the norm. The pulp's average solids content is 17%, of which approximately 53% is glucose and fructose (Kuti & Galloway, 1994). The rest (17% to 43% solids) is composed of proteins (0.21% to 1.6%), fats (0.09% to 0.7%), fibers (0.02% to 3.5%) and ashes (0.4% to 1%) (Sepúlveda & Sáenz, 1990). Prickly pear pulp is rich in ascorbic acid (40 mg per 100 g pulp) as well as other compounds such as calcium (15.4 to 32.8 mg/100 g), phosphorus (12.8 to 27.6 mg/100 g), and potassium (217 mg/100 g) (Sawaya et al., 1983; Sepúlveda & Sáenz, 1990). According to Batista et al. (2003), the carbohydrates that is possible to obtain from unstructured carbohydrates such as starches and pectins present in the *Opuntia* cladodes can be 640 g/kg, making it an important source of sugars.

Other main components of *Opuntia* spp. are the spines, which represent 8.4% of the total cladode. They composed of 96% polysaccharides, mainly cellulose (47.9%) and hemicellulose (48%) organized as fibers aligned along the spine, besides of the presence of lignin (1.2%) and 1.3% ash (Malainine et al., 2003) (Fig. 43.1).

In addition to the sugars present as the main components of the biomass of *Opuntia* spp., protein is one of the components of the cladodes. It can be present in the wet matter between 5–10 g/kg, while on a dry basis, it can find between 40–100 g/kg. These values may decrease depending on the age of the cladode (Stintzing & Carle, 2005). Loayza and Chávez (2007) observed a decrease in protein content from 0.94% in cladodes of 1 month of age to 0.48% in cladodes of 1 year of wet base age for *Opuntia ficus-indica*, var. Yellow. Another factor that can



**Fig. 43.1** Main components of *Opuntia* biomass. (a) thorns; (b) mucilage; and (c) fiber

**Table 43.4** Chemical composition of prickly pear peel (Habibi et al., 2009)

Constituent	(%)
Ash	11.5
Fats and waxes	11.0
Lignin	2.4
Protein	8.6
Mucilage	4.1
Other polysaccharides	35.0
Cellulose	27.0

affect the protein content in cladodes is the variety and cultivation conditions, Batista et al. (2003) found an amount of 62 g of total nitrogen per kg of biomass on a dry basis in 3 varieties of *Opuntia*.

Additionally, the presence of minerals such as calcium has been reported in amounts between 41–57 g/kg, an amount due to the absence of water and the high concentration of calcium in the soil. Magnesium can vary from 13–17 g/kg, phosphorus between 5–6 g/kg, copper between 8–9 g/kg, iron between 59–66 g/kg and manganese between 86–103 g/kg (Batista et al., 2003) as well as traces of cobalt and silica (Loayza & Chávez, 2007). For *Opuntia ficus indica* and *Opuntia amyclae*, high values have been found for Ca, K, Fe, Mn, and Zn (Abidi et al., 2009).

Regarding prickly pear fruit, the amount of skin has been reported to be 40% (wet weight basis) with 11% by weight of fats and waxes, as well as minerals (11.5%) in the skin, while the lignin content was 2.4% (Habibi et al., 2009). The main components of the skin are given in Table 43.4.

### 3 Biomass for the Production of Renewable Energy

The fuels generated by microorganisms and biomass are gaining acceptance as they are proven to be a technically and economically feasible alternative (Inrak, 2019). One of the advantages of using biomass and microorganisms to produce renewable energy is that it is possible to extract and/or produce not only energy but other



compounds of biological interest (prebiotics, fertilizers, materials rich in proteins, vitamins, lipids, etc.). Other industries such as food, forestry, agriculture, paper mills, and those that require specific organic inputs derived from the treatment of biomass and the products of its fermentation can be articulated. In addition to have an economic flow and materials, the waste from these industries can be used almost entirely, generating added values necessary to reduce production costs (Sheehan et al., 1998).

For the particular case of energy production from biomass, routes such as biochemistry and thermochemistry can be used, highlighting biogas and ethanol as generated products. It is also possible to obtain hydrogen, methanol, heavy oils, carbon, among other products, either directly or indirectly from sources such as animal excreta, bagasse, wood remains secondary crops, paper, grasses, and another type of lignocellulosic waste.

Traditionally, one of the methods to obtain energy from biomass is the direct burning, which must generally go through a drying process to increase its heat capacity. An example is the case of vaccine excreta, which contributes about  $1.5 \times 10^7$  J per kilogram of dry matter when incinerated. However, only 10% of these excreta corresponds to dry weight, which implies the evaporation of 9 kg water and a close investment of  $2.2 \times 10^7$  J for drying. In other cases, the burning of wood in water heaters has a very low efficiency (50%) compared to those that use gas. Thus, the direct burning of biomass could leave an alternative in the future to give way to the production of other fuels whose energy value can be fully exploited in rural areas.

On the other hand, the fermentation of biomass has been used for centuries to produce wine, beer, and distillates. This type of fermentation can also be applied to various biomass sources to produce fuels such as ethanol. The raw material used to produce this fuel has been sugar cane in Brazil and corn in the United States. In Mexico, it is not allowed to work with primary crops (corn, beans, wheat, etc.), which directly or indirectly used for human consumption. However, the remains of pruning, fruit, or their bagasse, molasses, and other secondary crops can be used.

To fragment these complex raw materials, hydrolysis should be carried out to obtain fermentable sugars. There are several methods to carry out hydrolysis of lignocellulosic material, but currently working in a combination of chemical and biological processes. Some hydrolysis types are carried out in acidic solutions, and specific enzymes are added to increase the release of sugars. It should be noted that an ethanol production from biomass requires energy and that, in this case, some percentage must be recovered, for example, from fermentation residues, which can be incinerated in the boiler. In addition to ethanol, other products such as acetone-butanol, acetone-ethanol, or butanol-isopropanol mixtures can be generated when the appropriate microorganisms are added. In addition to the fact that the aforementioned products have an economic value greater than ethanol, the amount of  $\text{CO}_2$  decreases in these biochemical routes, and this is important since  $\text{CO}_2$  is dispersed in the environment.

## 4 Treatments to Obtain Fermentable Sugars from *Opuntia* spp.

One of the main changes in the use of lignocellulosic residues for bioethanol production is the pretreatment to destroy the cellulose matrix and dispose of the fermentable sugars (Lipnizki, 2010). Lignocellulosic materials contain cellulose ( $(C_{10}H_{10}O_5)_x$ ) and hemicellulose ( $(C_5H_8O_4)_m$ ) that are bound to lignin [ $(C_9H_{10}O_3(OCH_3))_{(0.9-1.7)}]_n$  (Balat et al., 2008). Cellulose and hemicellulose are polymers built from long chains of sugar monomers (Pettersson et al., 2007). *Opuntia* spp. contains mucilage, which is a complex polysaccharide that contains a high water absorption capacity. It is mainly composed of arabinogalactans with sugars such as galactose, arabinose, rhamnose, and gacturonic acid (Peters et al., 2015). Additionally, it has been determined that the content of mucilages, pectins, and gums of *Opuntia* decreases by 27% with age, while the amount of insoluble fiber increases around 31% with age. In this insoluble fiber, cellulose is mainly included, hemicellulose and lignin (Rodríguez-García et al., 2007).

Cellulose chains are composed of a homo polysaccharide composed of  $\beta$ -D-glucopyranose units linked by (1–4) glucosidic bonds and intertwined in such a way that neither water nor enzymes can penetrate. *Opuntia* spp. is composed of 13% cellulose (Kuloyo et al., 2014; Yang et al., 2015), of which crystalline cellulose (16.6%) and amorphous cellulose (27.2%) are two of the constituents that have been determined. The higher amount of amorphous cellulose indicates that it is more susceptible to hydrolysis compared to crystalline cellulose (Yang et al., 2015). On the other hand, hemicellulose is a mixture of polymerized monosaccharides such as glucose, mannose, galactose, xylose, arabinose, and galacturonic acid as a connection between the fibers of cellulose and lignin. These sugars can be rapidly hydrolyzed with acid or base or with hemicellulase enzymes. Lignin is highly branched in structure and often highly resistant to conversion by microorganisms and chemical agents. Lignin is present in wood around 20 to 40% and various plant species such as bagasse, cob, peanut shell, rice pod, and straw (Talebniya et al., 2010; Balat et al., 2008). At the same time, *Opuntia* is in the range of herbaceous substrates of 9–18% compared to the lignin content of wood, which could benefit in generating recalcitrant compounds for the production of biofuels (Yang et al., 2015).

The conversion of cellulose and hemicellulose into sugar monomers such as carbohydrates of 5 and 6 carbons is complicated. Bioethanol production requires pretreatment and hydrolysis (Lipnizki, 2010), since the pretreatments disorganize the composition of the biomass, making it more accessible to cellulose to enzymatic hydrolysis processes allowing the breaking down of cellulose into simple sugar molecules (Udeh & Erkurt, 2017).

## 4.1 *Chemicals Methods*

Among the chemical treatments, different chemical substances are used such as acids, alkali, as well as oxidizing agents such as peroxide and ozone (Talebnia et al., 2010). Among chemical methods used, sulfuric acid, hydrochloric acid, nitric acid, phosphoric acid, and acetic acid are the most common. The acidic medium attacks polysaccharides, especially hemicellulose, which is easier to hydrolyze than cellulose (Cardona et al., 2010). However, the use of acid generates corrosion and decomposition of the hemicellulose sugars and requires the neutralization of pH (Talebnia et al., 2010).

Regarding alkaline treatment, it is used at low temperatures and pressure to be used at ambient conditions. The only limitation in the alkaline treatment is that some alkaline compounds are converted to salts or incorporated into the biomass. Alkaline treatments reduce the content of lignin and hemicellulose, allowing the passage of water molecules between the layers causing the bonds between hemicellulose and lignin to break (Balat et al., 2008).

## 4.2 *Physicists and Physicochemical Methods*

Since the lignocellulosic material does not contain sugars available for bioconversion, pretreatment is necessary to remove lignin and hemicellulose to increase porosity (Balat et al., 2008). An initial step is the reduction of particle size through grinding, pulverizing, crushing. Although it is an efficient initial treatment, its use is limited due to the high energy consumption to carry out the process due to the required particle size and the moisture present in the lignocellulosic material.

Other physicochemical methods used for the pretreatment of lignocellulosic material are water at 150 °C, since the solubility of some of its components depends on temperature and moisture content. Depending on the temperature, there are hydrothermal treatments in which the water is kept in a liquid state at high temperatures or the self-hydrolysis carried out through high-pressure water vapor, which causes a breakdown of the structure of lignocellulosic matter (Balat et al., 2008; Talebnia et al., 2010).

## 4.3 *Enzymatic Methods*

Enzymatic methods are based on using different microorganisms for the degradation of the different components of lignocellulose (Buranov & Mazza, 2008). The use of the enzymatic pathway is a promising way to obtain fermentable sugars. Many fungi such as white rot fungi produce oxidative enzymes such as laccase or lignin peroxidase, capable of degrading lignin, obtaining products such as vanillin,

dehirodivaniline, vanillic acid, ferulic acid, among other fragments of lignin (Buranov & Mazza, 2008). On the other hand, microorganisms such as *Trichoderma reesei* produce a set of cellulases such as cellobiohydrolase, endoglucanases, xylanases  $\beta$ -glucosidases, which are necessary to make the hydrolysis of cellulose efficient (Tabka et al., 2006).

Given the structural complexity of the lignocellulosic material, pretreatments can be combined to improve hydrolysis yields by maximizing the use of the lignocellulosic material (Sun et al., 2016). Among the commonly used combinations are enzymatic acid treatments that complement chemical treatments (Dagnino et al., 2013; Sun et al., 2016). To completely degrade cellulose with an enzymatic treatment, enzymes' synergistic action with different mechanisms of action is necessary (Fenila & Shastri, 2016). For the degradation of cellulose, a group of enzymes called cellulases is required including endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.176), exoglycohydrolases (EC 3.2.1.74), and  $\beta$ -glucosidases (EC 3.2.1.21). The degradation of hemicellulose requires a more complex group of enzymes including endo- $\beta$ -1,4-xylanase (EC 3.2.1.8),  $\beta$ -xylosidase (EC 3.2.1.37), L-arabinofuranosidase (EC 3.2.1.55),  $\alpha$ -glucuronidase (EC 3.2.1.139),  $\alpha$ -galactosidase (EC 3.2.1.22), acetyl xylan esterase (EC 3.1.1.72), and ferulic acid esterase (EC 3.1.1.73) (Carvalho et al., 2013; Maitan-Alfenas et al., 2015). The combination of enzymatic treatments after pretreatments can be influenced by several factors that might be classified into two groups: factors related to the enzyme and factors related to the substrate (Alvira et al., 2010; Ju et al., 2013).

#### 4.4 Hydrothermal Treatment

An alternative treatment for rapid hydrolysis of *Opuntia* flour is to use high temperatures and short exposure times. Texco-López et al. (2018) designed a hydrothermal treatment of *Opuntia ficus indica* flour using temperatures from 150 to 205 °C within 1, 5, and 10 min. It was observed that at 5 min and temperatures of 175 and 185 °C, the highest amounts of reducing sugars were obtained. In this type of treatment, high temperatures allow the water to be ionized, generating an acid environment that can favor the hydrolysis of mucilage, hemicelluloses, and cellulose (Carvalho et al., 2009). However, at higher temperatures, auto condensation, and the degradation of free carbohydrates significantly reduce fermentable sugars (Baêta et al., 2016). To promote hydrolysis in this type of treatment, it is possible to add small amounts of H<sub>2</sub>SO<sub>4</sub> (0.5–1%) that can increase the release of monosaccharides by up to 520% (Kim et al., 2016). The addition of acid and combined at high temperatures increases the concentration of H<sup>+</sup> ions that actively participate in the hydrolysis of all the biopolymers that make up the *Opuntia ficus indica* flour.

## 5 Effect of Cladode Age, *Opuntia* Variety, Temperature, and Solid Content on Fermentable Sugars Release

Studies have been carried out to analyze the composition of cladodes of *Opuntia ficus indica* based on their age and variety (Pérez-Cadena et al., 2018). The proximal analysis of different varieties (Copena VI, Giant Blue, Goliath, Atlixco, Energy, and Milpa Alta) was carried out and the results obtained are given in Table 43.5.

An important aspect of the composition of the *Opuntia* cladodes of the evaluated varieties was the content of holocellulose, which comprises the amount of cellulose and hemicellulose. It was observed that the amount of holocellulose was high in the variety Azul Gigante at 6 months (30%), while at 12 months this amount was lower (23.1%). The amount of lignin showed an increase, from 6.7 to 10%, which suggests that with the increase in the maturity of the cladodes, the concentration of lignin increases, probably because the cladode requires greater support from the plant, increasing its hardness (lignin) and amount of fiber (holocellulose). Similarly, it was observed that the ashes do not have a significant increase between the two ages, with an average of 20% in the evaluated varieties. Malainine et al. (2003) have found that the amount of ash present in samples of *O. ficus-indica* was 19%, in addition to 7.2% extractable in solvents and 3.6% by weight of lignin. On the other hand, Rodríguez-González et al. (2014) found amounts less than 15% ash in the mucilage obtained from six *Opuntia* varieties (*O. streptacantha*, *O. hyptiacantha*, *O. ficus-indica*, *O. tomentosa*, *O. joconostle*, and *O. atropes*). Aquino et al. (2012) found up to 4% ash in the fibers obtained from the *O. ficus-indica* cladodes. The results suggest that mucilage is the main source of ash followed by fiber, in such a way that the amount was similar in the two *Opuntia* ages evaluated.

The temperature, the content of suspended solids during the chemical hydrolysis process, and *Opuntia* varieties directly affect the release of free sugars. It has been observed that the high the amount of solids, and the rehydration process of *puntia* flour limits its solubility. This may be because the mucilage present in the cladode flour when rehydrated shows viscoelastic properties, an effect that is dependent on the concentration of the mucilage in the sample (Medina-Torres et al., 2000; León-Martínez et al., 2011), which causes a limitation in the homogenization of the initial reaction mixture for hydrolysis since the mucilage forms molecular aggregates (Cárdenas et al., 1997). According to Ginestra et al. (2009), the mucilage is present in both fruits and cladodes in around 14% in dry weight, whose main physiological function is to regulate the cell's water content and the calcium flux in the plant (Nobel et al., 1992).

On the other hand, it has been observed that temperature has a marked effect on *Opuntia* cladodes' hydrolysis. Idrees et al. (2014) observed that a maximum was obtained in the hydrolysis at high temperature and low residence times. The authors observed for a concentration of 1 and 2% sulfuric acid (3 h at 121 °C), 27.2, and 29.2 g/L were reached, respectively. The concentration of total reducing sugars increases when high temperatures and high reaction times are used. However, during their experimental work (Jeevan et al., 2011), they observed that although the

**Table 43.5** Percentage composition of *Opuntia* spp. varieties

	Moisture	Extractable in water	Extractable in solvent	Lignin	Holocellulose	Ash	
6 months	Copena V1	8.30 ± 0.27	47.75 ± 1.14	6.12 ± 0.65	3.85 ± 0.78	17.53 ± 3.01	27.58 ± 0.24
	Azul Gigante	9.29 ± 0.89	46.73 ± 4.96	5.41 ± 0.28	6.76 ± 0.54	30.06 ± 2.83	18.75 ± 0.07
	Goliat	8.95 ± 0.96	39.72 ± 1.82	6.47 ± 0.54	8.43 ± 0.15	16.90 ± 2.59	21.38 ± 0.23
	Atlixco	8.81 ± 0.70	46.28 ± 1.95	7.58 ± 1.21	6.62 ± 1.22	19.28 ± 3.40	17.75 ± 0.01
	Energy	8.01 ± 0.16	45.57 ± 0.42	6.43 ± 0.61	6.05 ± 0.60	21.19 ± 1.29	19.23 ± 0.69
	Milpa alta	8.33 ± 0.06	50.38 ± 2.93	2.60 ± 0.24	2.58 ± 0.00	18.53 ± 1.76	14.57 ± 0.12
12 months	Copena V1	8.40 ± 0.27	38.45 ± 2.49	5.80 ± 0.55	5.10 ± 0.45	13.60 ± 0.77	27.82 ± 0.50
	Azul Gigante	8.12 ± 0.89	43.48 ± 7.96	9.29 ± 1.14	10.02 ± 0.75	23.15 ± 2.96	19.95 ± 0.52
	Goliat	8.44 ± 0.96	40.91 ± 2.25	8.44 ± 1.01	10.84 ± 1.87	17.26 ± 1.48	22.47 ± 0.18
	Atlixco	7.17 ± 0.70	38.35 ± 3.23	8.78 ± 1.40	6.59 ± 0.28	15.68 ± 1.85	18.05 ± 0.25
	Energy	8.68 ± 0.16	40.84 ± 3.37	7.93 ± 1.54	8.84 ± 1.66	15.42 ± 0.27	21.64 ± 0.37
	Milpa alta	7.81 ± 0.06	43.49 ± 3.83	7.97 ± 0.57	12.46 ± 0.94	21.99 ± 1.36	18.62 ± 0.08

reaction was carried out for a long time, an increase in the amount of sugars was not observed, but a slight increase in the amount of inhibitory compounds.

When the content of solids is increased to more than 10% during hydrolysis, the amount of reducing sugars decreases. This may be because, during the acid treatment, the destruction of the structure of the lignocellulosic material is favored, releasing only the sugars present in hemicellulose without directly affecting lignin and cellulose (Silvério, 2013); in such a way that only hemicellulose and mucilage are the main biopolymers that hydrolyze at concentrations greater than 10% solids content.

It has been observed that age and variety are important factors in the amount of reducing sugars released during chemical hydrolysis. Pérez-Cadena et al. (2018) observed that on average, there is an increase of 20 to 30 g/L of sugars depending on the *Opuntia* variety and an increase in the concentration of sugars from 25 to 35 g/L depending on ages (6 and 12 months). On the other hand, Ribeiro et al. (2010) observed that there were significant differences in the amount of sugars for the varieties of cladodes analyzed, finding up twice as many sugars present in quaternary cladodes obtained from dry and rainy harvest periods. This effect is mainly because the bilateral network of lignocellulosic tissue of *Opuntia* increases as it matures, increasing its rigid constitution and intensifying its color (Aquino et al., 2012).

Another important aspect to consider in the extraction of sugars by the acid hydrolysis is the presence of phenolic compounds that originate from the degradation of lignocellulosic matter during chemical hydrolysis. Guevara-Figueroa et al. (2010) evaluated the content of phenolics and flavonoids of 12 *Opuntia* varieties (commercial, and wild). They observed that for the wild varieties purple, tempranillo, and crystalline, the concentrations of phenolic compounds were 19.9, 19.4, and 17.8 mg EAG/g. Santos-Zea et al. (2011) evaluated the content of phenolic compounds in 9 *Opuntia* varieties, obtained by an extraction process followed by hydrolysis. They observed that five varieties presented the same amount of phenolic compounds in value of 905.1 µg EAG/g. This difference may be mainly due to the process of obtaining phenolic compounds. Some phenolic compounds, such as *p*-coumaric acid, 4-hydroxybenzoic acid, caffeic acid, salicylic acid, and gallic acid, have been identified in *Opuntia* samples (Guevara-Figueroa et al., 2010). Other compounds including flavonoids and phenolics have been reported (Stintzing & Carle, 2005). Guevara-Figueroa et al. (2010) determined the content of phenolic compounds in lyophilisates of ten varieties of *Opuntia* and found that the amount of phenolics was from 2 to 20 mg/g.

## 6 *Opuntia* as Biofuel

Within the energy systems in which biomass is used, the main interest is obtaining ethanol, either by a biochemical or thermochemical route. There are several reasons why biomass is viewed favorably for energy production. In general, it can contribute

to sustainable development and it is usually available in the locality where a transformer plant would be installed. This transformation is more economical than the installation of an oil refining plant and, in environmental matters, the promotion and care of a crop promote the reduction of greenhouse gases since  $\text{CO}_2$  is the food of vegetables. The production chain would require labor that would be located in rural areas, while the waste generated can be treated and transformed into by-products to give them added value, closing a cycle that is friendly to the environment (Lin, 2006).

*Opuntia* has been used as a source of renewable energy to produce biogas (Fig. 43.2), obtaining good yields (Ramos et al., 2014). As a liquid fuel (bioethanol), the idea dates back to 1923 when a publication proposed it as a fuel for airplanes due to the zero cost of this plant and the widespread of it in that country. From this experiment, few studies were developed. One of them was the investigation carried out by Retamal in 1987, in which ethanol was produced from fruits (prickly pear) and cladodes of *O. ficus-indica*. In this work, HCl 1 N was used for the pretreatment of the substrate and commercial cellulases for the hydrolysis of the cellulosic fraction. In recent investigations (Santos et al., 2016; Kuloyo et al., 2014), the need to extract the greatest amount of sugars from the cladodes of *O. ficus indica* is highlighted in order to have good ethanol yields through fermentation. In the production of second-generation biofuels, it is necessary to develop techniques that help to take advantage of this plant with a view to a bioindustry and integrate unit operations, and biotechnological processes use of *Opuntia* spp. and other lignocellulosic residues (Hamdi, 1997).

Regarding the production of ethanol (Table 43.6), there are reports aimed at the fermentation of the juice obtained from the *Opuntia* fruit (prickly pear) to obtain wine using microorganisms such as *Saccharomyces cerevisiae* and *Pichia fermentans* with a yield of 8.73% (v/v) of ethanol (Castellar et al., 2008; Rodríguez-Lerma et al., 2011). On the other hand, Turker et al. (2001) mention that it is possible to



Fig. 43.2 Biogas generating plant from *O. ficus indica*



**Table 43.6** Ethanol production from *Opuntia* spp.

Product	$Y_{p/s}$	Alcohol content	Microorganism	Reference
Bioethanol	–		<i>Candida shehatae</i>	du Preez et al. (1989)
Bioethanol	0.086 L/kg	1.4% w/v	<i>Saccharomyces</i> sp.	Retamal et al. (1987)
Bioethanol	0.33 g/g	19.6 g/L	<i>Kluyveromyces marxianus</i> <i>Saccharomyces cerevisiae</i>	Kuloyo (2012)
Tuna wine	–	6.5 v/v	<i>Saccharomyces cerevisiae</i> <i>Pichia fermentans</i>	Gutierrez-Moreno et al. (2010)
Tuna wine	–	9.2% w/v	<i>Saccharomyces cerevisiae</i>	Lee et al. (2000)
Tuna wine	–	55.3 mL/L	<i>Saccharomyces cerevisiae</i>	Moßhammer et al. (2006)

obtain 55.3 mL ethanol from fermentation with *S. cerevisiae*; but it is necessary to establish optimization criteria for fermentation. Some criteria can be ethanol content, volatile compounds, organic acids, and compounds that produce color (Navarrete-Bolaños et al., 2013). Few studies focused on the use of *Opuntia* for the production of bioethanol due to the complexity of the processes and unit operations that must be used. Besides, there is a need for highly specialized microorganisms capable of fermenting pentoses and hexoses to obtain acceptable yield (Balat et al., 2008; Mendez-Gallegos, 2010).

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# Chapter 44

## *Opuntia* spp. in Biogas Production



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**Abstract** Safe environment and clean energy are among the most important green chemistry fundamentals. The world economy depends mainly on fossil fuel as a source of energy, and due to limited natural resources and uncontrolled pollution, the world is looking for alternatives that can meet the basic requirements of a clean and safe life. Using alternative sources such as biogas as an energy source can provide a solution to world energy problems. Biogas is produced through anaerobic digestion of organic matter, which is converted into combustible gas rich in methane. Biogas generation is one of the cheapest methods of producing energy on the spot from readily available resources. Nonedible agriculture crops and/or waste are among the essential biomass sources to produce biogas. Biomaterials produced from *Opuntia* species showed great potential as a source of biomass in the last years. Plants that belong to this genus can tolerate drought and poor soil fertility and require low maintenance. *Opuntia* biomass has been considered an excellent sub-

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strate alone or in combination with other organic waste materials. This chapter discusses the most recent research on *Opuntia*'s different species in biofuel production and their potential as an alternative energy source.

**Keywords** Environment · Biofuel · Biomass · Energy · Clean energy · Green chemistry

## Abbreviations

AD	Anaerobic digestion
CH <sub>4</sub>	Methane gas
kWh	Kilowatt per hour
L ha <sup>-1</sup> yr <sup>-1</sup>	Liter per hectare per year
t ha <sup>-1</sup>	Tonne per hectare
TS	Total solids
VS	Volatile solid

## 1 Introduction

Uncontrolled use of chemicals leads to drastic climate changes, representing one of the most critical challenges facing the world economy and the future sustainable development agenda. The world tends to move towards a green economy to reduce the harmful effects of the current industrial activities, especially in the energy sector. The green economy aims to reduce environmental risks and ecological scarcities, aiming for sustainable development without degrading the environment. The energy sector relies on fossil fuel, and the global annual energy demand of over 12 billion tons of oil equivalent results in the emission of 39.5 Gigatons of carbon dioxide (Gt-CO<sub>2</sub>), and the annual CO<sub>2</sub> emission would increase in the future (Abas et al., 2015). The CO<sub>2</sub> emission could be minimized by decreasing fossil fuels and replacing it with renewable ones such as biomass or improvement of energy efficiency in all human activities in different ways. Biomass energy utilizes biological waste organic materials for the production of heat or electricity. Fuelwood, charcoal, and animal dung continue to be essential sources of bioenergy in many parts of the world, and, to date, wood fuels represent by far the most common sources of bioenergy (Ruane et al., 2010).

Biodegradable waste could be an excellent source to produce useful energy, and at the same time leading to waste minimization. The waste treatment was recognized to produce biofuel and electricity introduced in many countries. The feedstock's organic fractions are degraded mostly into CH<sub>4</sub>, CO<sub>2</sub>, and digested residues (Barbosa et al., 2001). Production of methane-rich biogas through anaerobic



digestion of organic waste materials provides a versatile carrier of renewable energy, as methane can be used in replacement for fossil fuels in both heat and power generation and as a vehicle fuel, thus contributing to mitigate the emissions of greenhouse gases and slowing down the climate change.

Approximately 50% of the total energy consumed in Africa is obtained from fuelwood. However, there are several disadvantages related to this type of fuel, including adverse health effects resulting from the accumulation of indoor smoke, deforestation, and the time-consuming process of wood collection, which is dangerous from an environmental point of view and labor-intensive (Roopnarain & Adeleke, 2017). It was reported that the biogas project was proposed in Namibia is the digestion of the common reed (*Phragmites australis*), and recently, two small-scale biogas projects on a water treatment plant and a landfill have already been initiated in Namibia. Both are the Clean Development Mechanism (CDM) projects (Roopnarain & Adeleke, 2017).

## 2 *Opuntia* Distribution and Uses

The *Opuntia* genus (*sensu stricto*) is endemic to America. It belongs to the Cactaceae family and includes over 181 species comprising, based on morphological traits, a total of 29 series (Guzmán et al., 2003). It is also well known as the cactus pear plant or nopal. Prickly pear is a fast-growing cactus growing in dry, arid, and rocky plaeid pasture land and degraded guarigues throughout North and South America. They are also distributed in the Mediterranean basin, Middle-East, South Africa, India, Thailand, and Australia. Some *Opuntia* species and cultivars are currently cultivated in several world regions in America, Africa, Asia, Europe, and Oceania (Labra et al., 2003). Many *Opuntia* species have been declared by some countries across Africa and Australia as invader plants (Brutsch & Zimmermann, 1993).

*Opuntia* species are drought-resistant fodder and easily growing with a little attention. *Opuntia* is used mainly for fruit production, and as a source of food for humans and animals. The tender stems (nopalitos) are consumed as greens in some countries such as Mexico. The young cladode is consumed as a vegetable, the cactus pear is consumed as fresh fruit, flowers, and mature cladodes low lignified are used as forage. The mucilages showed interesting biological activities and used for pharmaceutical and cosmetic products. Several pharmacological activities have been reported and showed interesting activities against different human pathologies (Peña-Valdivia et al., 2008; Sáenz et al., 2004; Cárdenas et al., 1997).

The crop for fruit production of *Opuntia* generates high amounts of residues, i.e., premature fruits and exceeding cladods, that are removed for increasing the plant vigour. Additionally, the limited uses in the food industry, low lignin content, and its high productivity of plant biomass in arid regions are important factors enabling the cultivation of prickly pear for energy purposes in semi-arid areas (Weiland, 2003). Different countries, including Brazil, Italy, Kenya, Botswana, South Africa, and Ethiopia, have implemented prickly pears on a large scale in wastelands. This can

enable bioenergy production without disrupting food supplies and hence sustainable energy supply for the future (Santos et al., 2016; Belay & Ali, 2018; Roopnarain & Adeleke, 2017). The cladodes are an excellent source of carbon and can be used to balance the digestion of protein-rich wastes such as slaughterhouse wastewater (Panizio et al., 2020).

### 3 Composition

The common raw materials used for the production of biofuel, e.g., sugar cane and straw from different crops consist of cellulose, hemicellulose, and lignin as main constituents, in addition to small amounts of phenols, proteins, minerals, starch, fatty acids, oils, waxes, tannins, and resins. The biomass from prickly pear cladodes has a different chemical structure from previously mentioned materials with high pectin levels and relatively low levels of cellulose and lignin (Sáenz et al., 2004). The non-fibrous carbohydrates are the most important portion of prickly pear biomass, mainly represented by soluble sugars, polysaccharides, organic acids, and other types of reserve carbohydrates pectins. The chemistry of *Opuntia* is not fully covered; however, different secondary metabolites have been reported. Cladode chemical composition such as carbohydrates 64–71%; 3–7% (dry/wet basis), lipids 1–4%; 0.2% (dry/wet basis), and proteins 4–10%; 0.5–1% (dry/wet basis) showed variations depending on different factors. Younger cladodes show higher carbohydrate, protein, and water contents (Stintzing & Carle, 2005). Calcium oxalate, among other inorganic salts, contributes to the high ash content (8.5–23.7%) in *O. ficus-indica* cladodes (Contreras-Padilla et al., 2011).

#### 3.1 Sugar Content

*Opuntia* stems contain readily fermentable, water-soluble carbohydrates (3–7% fresh mass fractions). Total sugars amounted to 40–42% of the materials' dry biomass obtained by the direct drying of the fresh raw materials (Stintzing et al., 2005). Glucose is the major sugar; however, galactose, arabinose and xylose are present (Santos et al., 2016; Kuloyo et al., 2014; Yang et al., 2015). *Opuntia* fruit contains 6–14% (fresh weight), mainly glucose and fructose (Berat & Turker, 2005; El Kossori et al., 1998). To high monosaccharides' concentration in the biomass pre-treated with either yeast cells (fermentation) or chemical hydrolysis using acidic/basic conditions like HCl/NaOH at high temperature (80 °C) is required. However, these chemical methods decrease the water-soluble sugars due to chemical degradation (Calabrò et al., 2018).

### 3.2 *Mucilages and Pectins*

Both mucilages and pectins are complex carbohydrate polymeric structures and form colloidal solutions in water. They differ from each other in terms of the chemical structure and physical properties (Karawya et al., 1980). Extraction of mucilages takes place using mild conditions and under neutral conditions; however, pectins require heating for 2 h at different pHs. Mucilages have low uronic acid and high sugar content than pectins. Different studies demonstrated a high ratio of arabinose, xylose, and rhamnose (Goycoolea & Cárdenas, 2003).

Pectin is a carbohydrate fraction of biomass-derived materials and functions as an intercellular and intracellular cementing material. It is a polymer with a polygalacturonic acid backbone of  $\alpha$ -1, 4 linked D-galacturonic acid units. Sidechains usually contain neutral sugars, such as L-rhamnose, galactose, and arabinose. Besides, pectin may contain other sugars, like glucose, to improve its gel-forming properties (Bokelmann & Ryan, 1985). *Opuntia* total pectin has concentration range of 0.13% to 2.64% in wet basis (1% to 23.8% in dry-weight basis). *O. robusta* showed high pectin content (3.30/26.6 wet/dry basis) in comparison with *O. ficus indica* (1.91/13.8) (Villarreal et al., 1963). The full enzymatic hydrolysis of prickly pectins showed a mixture of galacturonate (56.3), galactose (6.5), arabinose (5.6), xylose (0.9), and rhamnose (0.5) (Stintzing & Carle, 2005).

The mucilages are responsible for the ability of the Cactaceae to retain water even under unfavorable climatic conditions (Saag et al., 1975; Goldstein & Nobel, 1991). The mucilage chemical structure contains variable amounts of neutral sugars such as arabinose, galactose, rhamnose, and xylose. The presence of galacturonic acid was also reported (Srivastava & Pande, 1974; Sáenz et al., 2004). The mucilage of *O. ficus indica* is composed of 24.6–42% arabinose; 21–40.1% galactose; 8–12.7% galacturonic acid; 7–13.1% rhamnose and 22–22.2% xylose (Trachtenberg & Mayer, 1981; Nobel et al., 1992).

### 3.3 *Other High-Molecular-Weight Compounds*

*O. ficus-indica* have high amorphous and paracrystalline (disordered) cellulose, and low content of crystalline cellulose, suggesting its lignocellulosic biomass would be even more readily hydrolyzable into fermentable sugars than would biomass from traditional herbaceous or woody feedstocks (Yang et al., 2015). The non-fibrous nature of cladode reflected from the prickly pear low content of cellulose (3%–13%), hemicellulose (up to 8%), and lignin (up to 14%). This represents a significant difference from lignocellulosic materials that commonly presents fiber content over 50%, with cellulose ranging from 25 to 50% (Ben-Thlija, 1987; Malainine et al., 2003). The lignin mass fractions in *O. ficus-indica* dry bagasses (12.3%) were substantially lower than those for woody feedstocks (21–32%; e.g., poplar, eucalyptus, and pine; 20–30% sugarcane). Feedstocks with reduced lignin mass fractions might

display reduced recalcitrance during biofuel production (Ragauskas et al., 2014; Cardona et al., 2010).

## 4 The Potential of *Opuntia* as a Source of Bioenergy

Prickly pear biomass can be converted by anaerobic bacteria to produce biogas. Anaerobic digestion is a complex biochemical process of degradation of organic matter in the absence of oxygen, and it is intended for the stabilization of organic matter to produce biogas and biofertilizer. Biogas generated in the process usually contains about 60% CH<sub>4</sub>, 35% CO<sub>2</sub>, and 5% a mixture of H<sub>2</sub>, N<sub>2</sub>, NH<sub>3</sub>, H<sub>2</sub>S, CO, O<sub>2</sub>, and volatile amines. This gas can be used as fuel to generate thermal and electrical energy. The resulting biofertilizer has essential plant nutrients such as N, P, K, and micronutrients that can be applied to the soil as fertilizer.

The average biomass productivity of prickly pear was studied on a large production scale in five locations in Brazil as one of the parameters to estimate biofuels' potential production. The study included two varieties of *O. ficus indica* (palma gigante and redonda) and *Nopalea cochenillifera* ("palma miúda"). The average fresh biomass productivity was nearly 90 t ha<sup>-1</sup> yr<sup>-1</sup> (Santos et al., 2016). The same group estimated the potential of biogas production from prickly pear biomass theoretically using the average dry biomass productivity of 7.9 t ha<sup>-1</sup> yr<sup>-1</sup>, the conversion rate of volatile solids (VS; 91%) of 517 m<sup>3</sup> t<sup>-1</sup> VS<sup>-1</sup> 3717 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup> of biogas is expected to be generated. The potential of electricity generation with biogas was theoretically expected, and an average of 4646 kWh ha<sup>-1</sup> yr<sup>-1</sup> was also estimated for electrical energy. This amount (from 1 ha of prickly pear) could produce biomass to generate electricity sufficient to meet the annual consumption of about two homes (Santos et al., 2016).

Obach and Lemus (2006) estimated high production (23,400 kWh ha<sup>-1</sup> yr<sup>-1</sup>). The author's calculation was based on the average production of 300 ton ha<sup>-1</sup> yr<sup>-1</sup> production of 58 m<sup>3</sup> t<sup>-1</sup> SV<sup>-1</sup> of biogas (with 52% methane) and a generation of 1.5 kWh m<sup>-3</sup> biogas. Another similar and large-scale study conducted in Italy, cultivation of 159 10<sup>3</sup> ha prickly pear, produced an average of 3849 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup> of biogas from which ~342 ton ha<sup>-1</sup> yr<sup>-1</sup> of biomethane could be extracted, or 67,038 MWh of electric energy and 70,390 MWh of thermal energy could be generated. The data was close to the second study by Obach and Lemus (Compartmentia et al., 2017). These values were very close to traditional energy crops such as maize (5780 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup>), alfalfa (3995 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup>), and fodder beet (5800 m<sup>3</sup> CH<sub>4</sub> ha<sup>-1</sup>) (Weiland, 2003).

*O. ficus-indica* biomass was evaluated for biogas production in Kenya. The plant was cultivated on a large scale under various planting densities on marginal land blocks (900 m<sup>2</sup>/experiment). The methane production varies between 578 and 1860 m<sup>3</sup> per ha, depending on planting density during a 4-months growth period (Krümpel et al., 2020).

## 5 Optimization of the Biogas Production

The biogas production efficiency from the cladods biomass alone using AD are low when compared to co-digestion. Mixing the cladods with animal manure improved the biogas production (Uribe et al., 1992; Varnero et al., 1992; Varnero & López, 1996; Varnero & García de Cortázar, 1998). The biogas production of slaughterhouse wastewater was improved by co-digestion of the of slaughterhouse wastewater (75%) with *O. ficus indica* (25%) under semi-continuous conditions (Panizio et al., 2020). The green algae *Scenedesmus* sp. biomass is not an adequate substrate for anaerobic digestion due to its low biodegradability and low biogas yield. The co-digestion of *O. maxima* (75%), and *Scenedesmus* sp. (25%) based on VS content improved methane yield compared to both substrates' mono-digestion yielding up to 308 L CH<sub>4</sub> kg VS<sup>-1</sup>. Co-digestion enhanced the anaerobic digestion process and avoided inhibiting the low C/N ratio of microalgae (Ramos-Suárez et al., 2014).

*O. ficus indica* is an ideal plant for African arid regimes as a potential bioenergy source. A short acidic aerobic pretreatment (9 h) of *O. ficus indica* biomass before anaerobic digestion yielded 123% higher methane yield when compared to that without pretreatment. The aerobic pretreatment suggests that there was increased hydrolysis with retreatment (Myovela et al., 2019). The results were further supported by Calabrò et al. (2018) and showed enhancement of methane production using the acidic pretreatment (HCl) compared with thermal and alkaline treatments.

*O. ficus-indica* grows abundantly in the northern part of Ethiopia. The co-digestion of *O. ficus indica* fruit peel with cow dung in a ratio of 1:3 under mesophilic conditions (38 °C) using batch digester showed enhancement of biogas production (Gebrekidan et al., 2014). The pH has a direct effect on the quality of the produced biogas. The optimum conditions were found to be at pH = 6. The fermentation of different manure/prickly pear mixtures ratios showed the importance of maintaining a pH 6 or higher. At lower pH levels, CO<sub>2</sub> becomes dominant and the combustibility and energy content largely reduced. Increasing the proportion of manure in the mixture and using cladodes older than 1 year keep the neutral or basic pH, produce methane-rich biogas (methane content higher than 60%) (Varnero & López, 1996; Varnero & García de Cortázar, 1998).

## 6 Bio-Ethanol Production

Few studies have been conducted on the conversion of *Opuntia* biomass to bioethanol. The production of bioethanol from cactus biomass still beyond the expected level. The biomass's high-water content and the low total solids are the main reasons directly related to the low yield of bioethanol. The cladode biomass's viscosity, due to pectin presence, can be decreased using pectinase in the enzyme cocktail. A mixture of pectinase and cellulase enzymes was necessary for pectin solubilization, and the complete release of sugars (Grohmann et al., 1995). Enzyme mixtures

consisting of cytolase, pectinex, rapidase, and viscozyme were used to hydrolyze *Opuntia* cladode mucilage to improve its extractability and release of sugars (Kim et al., 2013). Pretreatment of fresh or dried *O. ficus-indica* cladodes with cellulase enzyme and acid slightly improved sugar release and ethanol yields above those of non-pretreated material; however, ethanol yields remained low at ~1.4% w/v (Retamal et al., 1987).

Under non-aerated conditions, separate hydrolysis (using 1.5% H<sub>2</sub>SO<sub>4</sub>) and fermentation of *O. ficus-indica* cladodes biomass using *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* resulted in similar ethanol yields of ~2.6% w/v (Kuloyo et al., 2014). In oxygen-limited cultures, *K. marxianus* exhibited almost double the ethanol productivity compared to non-aerated cultures. The obtained results showed an improvement over earlier attempts. The authors recommended hydrolysis of the biomass and to use of pectinase enzyme to reduce the viscosity of the slurry and increase the release of fermentable sugars to attain an economically viable ethanol yield of >4% w/v (Kuloyo et al., 2014; Wingren et al., 2003).

Hydrolyzed fresh and dried biomass had similar glucose, xylose, and galacturonic acid concentrations. The enzymatic hydrolysis using *Saccharomyces cerevisiae* (33 °C, 8 h) for the biomass from *Nopalea cochenillifera* and *O. stricta* (partially dried, 30% TS) produced ethanol 29.4 and 37.5 g L<sup>-1</sup> biomasses, respectively (Alencar et al., 2020). The estimated potential of ethanol production for the three prickly pear varieties studied in Brazil is in the range of 1490–1875 L ha<sup>-1</sup> yr<sup>-1</sup>. These values are lower than those reported for the main energy crops used for ethanol production, such as sugar cane (5300–9400 L ha<sup>-1</sup> yr<sup>-1</sup>), sugar beet (5000–6000 L ha<sup>-1</sup> yr<sup>-1</sup>) or cassava (3600 L ha<sup>-1</sup> yr<sup>-1</sup>) (Santos et al., 2016). However, prickly pear can produce ethanol in semi-arid zones, where those crops cannot be cultivated. Moreover, the water consumption per unit of ethanol produced from prickly pear cladodes should be lower for the prickly pear than other crops used today.

## 7 Conclusion

This chapter focuses on the production of biogas from the *Opuntia* plant. The process of anaerobic digestion of the *Opuntia* plant results in biogas production and a nutrient-rich digestate. In a broad spectrum, these species are well known for an extraordinary ability to produce biomass in soil and climate conditions, which are unfavorable for most plant species, in part due to their high water use efficiency. Soon, given the prospect of water scarcity, this fact must be evaluated by the biofuel industry. Furthermore, several studies are needed to be conducted and evaluated, more consistently, both the sustainability of biomass production for ethanol and biogas production, especially for the newly emerged prickly pear diversities.

For biogas production, despite its potential and significance to arid regions, very little research data is available about prickly pear anaerobic digestion, and more research has to be conducted. For ethanol production, it is necessary to enhance the

steps of biomass pretreatment, wherein pH must be near neutral for the application of biological processes, the high viscosity of the cladode biomass slurry should be reduced and the fermentable carbohydrate concentration in the hydrolysate should be increased. Hence it is important to produce an eco-friendly, economically viable process for ethanol concentration of at least 4% (w/v) from lignocellulose feedstock.

Due to global warming increasing in recent times, there is currently a huge demand for renewable energy and the prospects of more limited water resources, and the potential use of prickly pear cladodes for biofuel production deserves to be investigated. It faces numerous technological challenges at all stages of production. From an agricultural point of view, cultivation in the field requires much improvement in management practices for planting and harvesting systems, especially with the cropping operations' mechanization. The conversions of the biomass into biofuels still need ground breaking research.

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# Chapter 45

## *Opuntia* spp. in Dye-Sensitized Solar Cells



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**Abstract** Solar energy is one of the most important renewable and sustainable forms of energy. Different technologies have been developed to harness this free energy and contribute to the world economy, especially in countries with no seasonal variations in solar radiation. Dye-sensitized solar cells (DSSCs) are simply used to collect solar energy and convert that visible light into electricity. This depends on the photosensitization of wide band-gap metal oxide semiconductors. The photoelectrode spectral sensitivity needs specific chemical dyes for the lower energy photons collection. Significant research efforts for identifying new organic dyes from natural sources have been carried out. Higher plants contain many natural pigments well-known to interact with solar radiation. The fruit of the *Opuntia* spe-

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cies contains a particular type and unique pigment called betalain. This type of compound has an interesting conjugation system with nitrogen atoms forming an unusual natural dye. Betalain has a potential interest in the photosensitization process when added to the DSSCs system. This chapter provides a review on the structural features and potential applications of the betalain pigments in the photosensitization solar cells.

**Keywords** Solar energy · Photovoltaic cell · Counter electrode · Green nanotechnology · Dye-sensitized solar cells · DSSC · Betalain pigments

## Abbreviations

CE	Counter electrode
DSSCs	Dye-sensitized solar cells
FTO	Fluorine-doped tin oxide
HOMO	Highest occupied molecular orbital
HTMs	Hole transport materials
ITO	Indium-doped tin oxide
LUMO	lowest unoccupied molecular orbital
OPV	Organic solar cells
PCE	Power conversion efficiency
Ru	Ruthenium

## 1 Solar Cells

Solar energy is the most basic energy source that is free, clean, renewable, and an abundant alternative to reducing real-time dependency on traditional fossil fuels (Buscaino et al., 2008; Ammar et al., 2019). Over the past decade, the development of different types of solar cells has been deliberated. These cells were shaped to convert the visible light into electricity through the photovoltaic effect. Recently, different solar cells have been used extensively for various industrial applications and national domestic use (Dang et al., 2018; Schindler et al., 2018).

The solar cells are generally characterized based on their material composition into different types such as organic solar cells (OPV) (Steim et al., 2010); dye-sensitized solar cells (DSSCs) (Jamalullail et al., 2018); gallium arsenide based solar cell (Abdin et al., 2013), amorphous, multiple crystals or polycrystalline, and single-crystal silicon solar cells; where they reveal different efficiency values. The traditional solar cell is usually made of a silicon crystal material despite its high cost compared to OPV and DSSCs solar cell types (Adedokun et al., 2016). DSSCs are attracting great interest in terms of fabrication cost, design simplicity,

environmental friendliness, and performance (Abdel-Latif et al., 2013). However, their large-scale applications' operation is still limited due to long-term stability issues, which will also be covered with future improvement efforts.

### **1.1 Organic Solar Cell (OPV)**

Organic solar cells (photovoltaic cells) are promising cells that received much attention due to cheap cost architecture, characteristics, wide applications, and significant improvement in power conversion efficiency. Organic bilayer solar cells were reported for the first time in 1986 by Tang using complementary electron donor and acceptor organic molecules, exhibiting a power conversion efficiency close to 1% in sunlight (Abdulrazzaq et al., 2013). OPV revealed a substantial increase in stability, with reports providing evidence of the stabilities of 7 years. Compared to inorganic solar cells, OPV has the additional advantage that they can be processed at a modest temperature from solution, without the need of a vacuum, and by fast roll-to-roll processes. The organic photoactive materials used in organic solar cells show high absorption coefficients, which implies that only a very thin photoactive layer (100 to 200 nm) is required to absorb sufficient light. The recent developments give increasing credence that OPV can be a viable alternative technology to the present inorganic solar cells (Cao & Xue, 2014).

### **1.2 Construction and Working Principle of Organic Solar Cells**

Organic photovoltaic solar cells reveal a heterostructure of two different semiconductor types. The heterostructure is sandwiched between two functional electrodes for collecting the electrons and holes separately and is seated on a thin substrate of glass or PET. All devices need to be encapsulated to eliminate water and oxygen exposure (Cao & Xue, 2014; Kippelen & Brédas, 2009; Mazzio & Luscombe, 2015). The interaction of the photovoltaic cells with the light creates an excitonic cell. The electron is stimulated in either the donor or the acceptor from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) (Osman, 2017). Orbitals bandgap energy defines the possible light wavelength(s) that can be absorbed.

OPV cells have a limitation in the large scale production attributed to the devices' stability and lifetime in addition to the power conversion efficiency (PCE) (Scharber & Sariciftci, 2013). The PCE could be enhanced by increasing the absorption of light. However, there is a limitation on the absorption due to the active layer's thickness control up to 100 nm maximum. That thickness restriction comes from the short lifetime of the excitons and the conducting layer's low mobility. Metallic

nanoparticles have been used to enhance the absorption without increasing the active layer thickness (Kour & Mehra, 2017; Scharber & Sariciftci, 2013).

## 2 Dye-Sensitized Solar Cell (DSSC)

DSSCs are unique photovoltaic third-generation devices known as Grätzel cells. O'Regan and Grätzel (1991) reported the DSSC for the first time through sensitizing titanium dioxide NPs films with ruthenium bipyridyl complex (Jamalullail et al., 2018). It is commonly well known as low-cost cells due to their materials composition and low fabrication cost. These new solar cell types mainly convert solar energy (photons) into electrical energy through the sensitization of a wide-band-gap semiconductor (Sharma et al., 2018). Different parameters, such as voltage, current density, and conversion efficiency, are highly considered for the solar cell electrical performance. That performance relies totally on the solar cell dye type, which is generally categorized into inorganic and organic dyes (Jamalullail et al., 2018). One of the most common inorganic dyes with a high energy conversion efficiency (11–12%) in DSSCs is ruthenium complexes (Qin & Peng, 2012). Ruthenium (Ru) is one of the rare transition noble metals belongs to the platinum group metals. In addition to Ruthenium's high price (Gunn, 2013), its toxicity limits its use in many fields. Even though the Ru complex dyes and others like osmium complexes reveal high stability and efficiency, their toxicity, rarity, and fabrication cost eliminate their use in the DSSCs. A new research study on green extracted organic (natural) dyes has received much attention recently (Mansour, 2018). Many reports confirmed the efficiency of different natural dyes such as betalain (Isah et al., 2015; Ramamoorthy et al., 2016; Zhang et al., 2008), anthocyanin (Ramamoorthy et al., 2016), and chlorophyll (Arof & Ping, 2017; Syafinar et al., 2015), in supporting the DSSC electrical performance. Using such natural pigments is attracting much interest from an economical and environmental aspect.

### 2.1 Structure of DSSCs

DSSCs have a different basic structure compared to other PV solar cells, which rely on solid-state semiconductor materials, while DSSC rely on a combination of solid and liquid phases. The DSSC consist of transparent and conductive substrates (to allow sunlight to reach the cells), working electrodes (a semiconducting oxide, e.g., TiO<sub>2</sub>), dye (photosensitizer; to absorb the light), an electrolyte, and a counter electrode (CE). To understand the DSSC structure, a general working concept of the DSSC could be summarized as soaking a specific working electrode with a dye and sealing it to the counter electrode soaked with a thin layer of electrolyte (Sharma et al., 2018).

### 2.1.1 Transparent and Conductive Substrate

Based on the DSSC components, the DSSC mainly has two electrodes. One electrode should use a transparent substrate for the visible light and be considered an anode (Bauer et al., 2002). That transparent electrode should also have a high conductivity characteristic to eliminate the loss of the transferred energy. The most commonly used conductive substrates are fluorine-doped tin oxide (FTO,  $\text{SnO}_2$ : F) and indium-doped tin oxide (ITO,  $\text{In}_2\text{O}_3$ : Sn). These transparent conductive oxides are usually used to cover the glass. While, FTO reveals ~75% transmission efficiency, the ITO shows >80% transmittance in the visible solar light (Hug et al., 2014; Ludin et al., 2014).

### 2.1.2 Working Electrode Construction

FTO or ITO transparent conductive substrates are used as a base for further deposition of thin layer oxide semiconducting material with a wide band-gap. That layer has a typical thickness of 10 $\mu\text{m}$  with a 50% porosity. Different oxides could be used, however  $\text{TiO}_2$ ,  $\text{ZnO}$ , and  $\text{Nb}_2\text{O}_5$  are the preferred photo-electrodes (Jose et al., 2008).

### 2.1.3 Dye (Photosensitizer)

The dye is the third component in the DSSC structure, which plays a vital role in absorbing solar light and its conversion to electrical energy. The DSSC's performance depends on the dye type that acts as a sensitizer (Suganya & Jaisankar, 2019). According to Qin and Peng (2012), any selected dyes for DSSC should have the following specific physical and chemical properties:

1. High absorption performance of the light under both the ultraviolet-visible (UV-vis) and near-infrared region (NIR)
2. High adsorption ability to the semiconductor surface ( $\text{TiO}_2$ ) of the working electrode
3. High ability for efficient injection of the electrons into the conduction band of the semiconductors
4. Have characteristic luminescent properties
5. Have a hydrophobic property to enhance the DSSC stability by reducing the contact between the anode and electrolyte
6. Electro and photo-chemically stable.

Moreover, to the previous features, for more efficient stability, additional co-absorbents such as chenodeoxycholic acid and alkoxy-silyl- (Fung et al., 2003), carboxylic- (Hagberg et al., 2008), and phosphoric acids groups (Zaban et al., 1998) should be placed between the dye and the semiconductor layer (e.g.,  $\text{TiO}_2$ ) to avoid dye aggregation above that layer and decrease the recombination response between

the electrons and electrolyte (Neale et al., 2005). Accordingly, dyes were categorized into two types: inorganic and natural organic dyes.

### 3 Electrolyte

The electrolyte has an essential role in charge transfer between the anode and the counter electrode and should also enhance the connectivity between them. It contains a redox couple, solvent, additives, ionic liquids, and cations. High dielectric properties, low viscosity, high boiling temperature, non-corrosive, non-volatility, chemical, thermal, and electrochemical stability are properties for the optimum electrolyte (Gorlov & Kloo, 2008). The electrolyte spectra should not overlay the dye spectra, while the redox couple, such as  $I^-/I_3^-$ , should restore the oxidized dye efficiently (Sharma et al., 2018).

### 4 Counter Electrode (CE)

The counter electrode is an essential part of the DSSC structure since it is responsible for transferring the electrons from the external circuit back to the redox couple electrolyte. It collects the holes from the hole transport materials (HTMs). It should therefore have a conducting capability to transport the current through the DSSC solar cell. Counter electrodes are mainly made of platinum (Pt) or carbon (C). However, Pt is the most effective material due to its high performance in  $I^-/I_3^-$  liquid electrolyte reduction process (Adedokun et al., 2016).

## 5 Dye Types

### 5.1 Inorganic Dyes

The most important feature for determining DSSC efficiency is the rate of absorption efficiency of the solar light and dye anchorage to the semiconductor working electrode cell (Lee & Yang, 2011). The best example is ruthenium complexes, which showed good light-harvesting efficiency (around 12%), outstanding stability, good electrochemical properties, and a wide range of light absorption (Hernández-Martínez et al., 2012).

## 5.2 Natural Organic Dyes

Natural dyes are eco-friendly, cheap, non-toxic, widely available, and biodegradable with a high ability to reduce noble metals instead of using chemical reducing agents (Calogero et al., 2013). Therefore, they have received attention for use as photosensitizers in the DSSC fabrication as an alternative to the synthetic ones. Different natural dyes can simply be extracted from the plant's different parts (Adedokun et al., 2016). The nature of these pigments can impact their conversion efficiency performance (Narayan, 2012). Several studies reported the ideal conditions of DSSC operation and the possible useful dyes from different plants (Godibo et al., 2015).

Different natural dyes such as anthocyanin, betalain, betaxanthin, bixin, carotenoid, chlorophyll, crocetin, crocin, cyanine, flavonoid, lutein, mangostin, neoxanthin, rutin, violaxanthin, and tannin were tested (Hug et al., 2014; Ludin et al., 2014; Calogero et al., 2015; Ghann et al., 2017; Atli et al., 2019; García-Salinas & Ariza, 2019; Kabir et al., 2019; Sreeja & Pesala, 2019; Duan et al., 2020; Ishak et al., 2020; Onah et al., 2020; Sabagh et al., 2020; Zhang et al., 2020). Among those different tested natural dyes, betalains, anthocyanins, and chlorophylls were the most promising natural dyes for DSSCs.

## 6 Betalains

Betalains are nitrogen-containing plant pigments classified under alkaloids and different from the commonly known plant pigments anthocyanins. These pigments are divided into two main structural groups, betacyanins (red-violet pigments) and betaxanthins (yellow pigments) (Fig. 45.1). Betalains are found in the plant leaves, flowers, stems, and bracts (Gandía-Herrero et al., 2016) of certain families of flowering plants such as Cactaceae. Both betacyanins and betaxanthins possess a

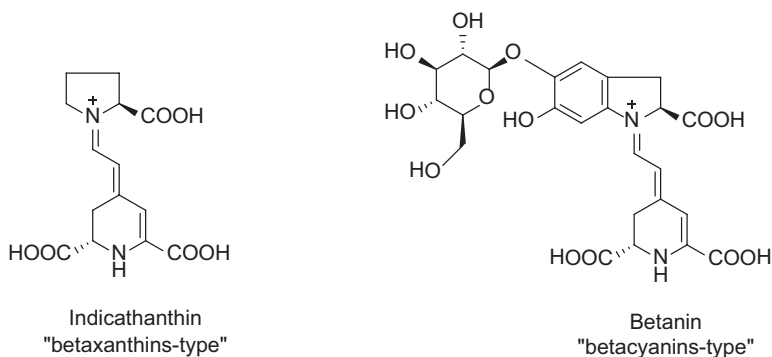


Fig. 45.1 Structure of betanin and indicathanthin



dihydropyridine moiety attached *via* a vinyl group to another nitrogenous group. The basic chromophore of the betalain pigments is the 1,7-diazaheptamethinium system (Clement et al., 1994). *Opuntia* genus belongs to the Cactaceae family and is considered to be the most promising source of betalains (Castellar et al., 2008; Calogero et al., 2010).

Betalains are water-soluble pigments that absorb the light in the visible region (Ludin et al., 2014). It has a strong solar absorption efficiency in the range of 400–600 nm. Betacyanins absorb the light at  $\lambda_{\max} = 536$  nm, and betaxanthins absorb at wavelength  $\lambda_{\max} = 480$  nm (Miguel, 2018). These two structures are easily found in *Opuntia* (mainly *Opuntia ficus indica*), the dragon fruits from *Hylocereus* cacti (mainly *Hylocereus polyrhizus*), the Swiss chard, and red beetroots (*Beta vulgaris*) (Azeredo, 2009; Rodriguez-Amaya, 2018). Due to the hydrophilic characteristic of betacyanins and betaxanthins, betalains are accumulated in the epidermal and sub-epidermal plant tissues (Wink, 1997).

Betalain binds easily to the semiconductor layer ( $\text{TiO}_2$ ) of the working electrode through its carboxylic group. As much as the dye molecules are attached to the  $\text{TiO}_2$  layer, the DSSC will equally gain higher energy conversion efficiency. Betalain can also effectively interact with the redox couple in the electrolyte (iodine/iodide) (Sandquist & McHale, 2011).

## 6.1 Betalain for DSSC

Zhang et al. (2008) reported the efficiency of using the betacyanin in DSSC, where it reveals the energy conversion efficiency of around 0.67%. Calogero et al. (2010) did a comparative study between the betalain and the synthetic Ru-complex common dye, N719. The study revealed a 1.7% energy conversion efficiency for betalain, which was less than the Ru dye. The thermal stability of the natural pigment was confirmed under solar irradiation. However, no confirmation was reported on the long term stability and the lifetime of the betalain sensitized solar cell (Calogero et al., 2010). A better energy conversion efficiency of betalain based DSSC of 2.06% was obtained using betalains from wild Sicilian prickly pear (Calogero et al., 2012). An electrochemical and optical study of betalain as a sensitizer in the DSSC revealed the interaction effect of the carboxylic acid functional group of betalain with the semiconductor layer, which enhanced the conductivity of the cell. That effect of the functional group of the dye was demonstrated later in 2016 by Ramamoorthy et al., where betalain and anthocyanin were extracted from *Opuntia dillenii* and *Tamarindus indica* for DSSC fabrication. Both dyes revealed a sensitized effect to the semiconductor layer ( $\text{TiO}_2$ ) (Ramamoorthy et al., 2016). For betalain pigments, its carboxylic acid group connected to the hydroxyl group in the  $\text{TiO}_2$  layer, facilitating electron transfer by a coupling effect (Hernandez-Martinez et al., 2011), while for anthocyanin pigment, its carbonyl and hydroxyl groups attached to the  $\text{TiO}_2$  layer. Both dyes showed a UV absorption band at 525 nm. The betalain based DSSC revealed a higher energy conversion efficiency (0.47%) than anthocyanin

based DSSC (0.14%), attributed to their functional group's sensitizer effect. In comparison, the dye mixture in a 1:1 ratio showed a 0.20% energy conversion efficiency, which is higher than that of anthocyanin based DSSC and lower than betalain based DSSC.

Isah et al. (2015) extracted betalain dye from *Bougainvillea glabra* flower for DSSC fabrication. The effect of pH on the betalain dye as a sensitizer in the DSSC was tested under different pH values (1.2, 3.0, and 5.7). The results indicated that the best conversion efficiency of 0.9% was achieved at pH 3. When the pH was 1.2, the efficiency was very low (~0.2%), which could be due to betalain's degradation process at high acidity. Meanwhile, at a pH of 5.7, the efficiency was 0.57%, which was also less than the efficiency obtained at pH 3 due to lack of absorption of betalain on the TiO<sub>2</sub> layer (Isah et al., 2015). Gokilamani et al. (2015) reported betalain pigments' effect in sensitizing the TiO<sub>2</sub> annealed thin film in the DSSC. The betacyanin from *Basella alba* var. *rubra* spinach leaves was extracted and used to prepare a DSSC, which exhibited 0.70% energy conversion efficiency. This was attributed to the effective sensitive nature of the dye and the thin film crystalline structure (Gokilamani et al., 2015).

The co-sensitization effect of a mixture of two natural dyes on the DSSC semiconductor layer was tested by Kumar et al. (2016). It has been described as an effective strategy for harvesting more solar light effectively. Among different tested extracts, cactus (*Opuntia ficus indica*) and Bermuda grass (*Cynodon dactylon*) co-sensitized DSSC showed a 1.1% power conversion efficiency, which was higher compared to their mono sensitized DSSC (Bermuda ~0.1%, and cactus ~0.6%). Almost the same result as for the cactus dye was achieved by Ganta D. 2017, where cladodes of *Opuntia ficus indica* was tested as a sensitizer of DSSC. A comparison between *Aloe vera* dyes extract, and cladodes extract reveals a high conversion efficiency of about 0.74%, corresponding to cladodes dyes extract (Ganta et al., 2017). This is attributed to the adhesion of chlorophyll dye to the TiO<sub>2</sub> layer resulting in better charge transfer. Interestingly, a side-by-side alignment of the two dyes, which was reported to reduce the mixing of dyes, demonstrated a 0.50% conversion efficiency (Ganta et al., 2017).

In contrast, two different betalain sources (*Opuntia ficus indica* and *Beta vulgaris*) displayed low conversion efficiency compared to *Hylocereus undatus*, the source of anthocyanin dye (Suganya & Jaisankar, 2019). Purushothamreddy et al. (2020) extracted the betalain from *Opuntia ficus indica* grown in Western Ghats Mountain in the southern part of Taminadu, India. They characterized the prickly pear fruit extract for its optical properties using UV-Vis and FTIR to confirm the presence of betacyanin and explore its promising characteristics as a photosensitizer for use in DSSC (Purushothamreddy et al., 2020). They further fabricated a DSSC with a conversion efficiency ( $\eta$ ) of 0.56% with the highest fill factor (FF) of 0.85, open-circuit voltage (Voc) of 0.56 V, and short circuit-current density (Jsc) with 1.17 mA/cm<sup>2</sup>.

## 7 DSSC Stability Challenge

DSSCs are of great interest due to their fabrication simplicity, eco-friendliness, flexible design with a good energy conversion efficiency. They also open a new commercial window for a sustainable, cheap, and useful product that can compete with other types of solar cells. However, the main problem with this type of solar cell is the stability issues and purification process. DSSCs show less stability than the synthetic inorganic-complex-based cells, i.e., natural DSSCs show less efficiency in contrast with the artificial ones, which is mainly attributed to the weak binding with the TiO<sub>2</sub> semiconductor layer, wherein the dye structure impact the cell performance (Ammar et al., 2019). Some natural dye structures may reduce contact with the semiconductor layer and efficiently inhibit the array of the molecules on the semiconductor layer. This negatively affects the number of electrons reaching the conduction band in the TiO<sub>2</sub> layer, decreasing the light absorption intensity, and hence the solar cell's performance. The stability of DSSC is based on its components, such as the dye sensitizer, semiconductor layer, counter electrode, electrolyte, and the working electrode (Sharma et al., 2018). This is in addition to other parameters such as the semiconductor layer thickness, extraction solvent, the electrolyte, and the active area ratio (Ammar et al., 2019). Enhancing the long term stability of the DSSC is one of the exciting topics for motivating this new type of solar cell. The DSSC cell should be stable and at an acceptable performance level for around 20 years. However, the natural dyes degrade upon exposure to UV radiation. UV filters can cover the outer anode as an initial step for protection and filtration of the UV radiation to enhance the stability.

The stability of the redox electrolyte is also an essential parameter in the DSSC performance. Preventing the decomposition of the redox couple will enhance DSSC stability. Even the extraction solvent for the natural dye could impact the light absorption and stability of the DSSC (Aduloju et al., 2011). Besides, the effect of a fast injection and intense light absorption for DSSC performance enhancement has been reported (Aduloju et al., 2011). All these variable parameters are concerned with the stability issues of the DSSC and represent a real research challenge for its industrial applications.

## 8 Conclusion

Natural dyes are promising alternatives to synthetic dyes considering their natural origin and economic value. The low-cost production, simple preparation techniques, eco-friendliness, and availability of natural dyes are important features. Among different tested natural dyes, betalain revealed a high potential efficiency in DSSC, whereas *Opuntia* spp. is the most promising betalain source. Although the natural dyes' efficiency in the DSSC looks promising, low stability is one of the major issues for this type of technology.

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# Chapter 46

## Incorporation of *Opuntia* spp. into Food Systems



Bilge Taşkın and Zeynep Aksoylu Özbek

**Abstract** *Opuntia* spp. has widespread species which are well-adapted to arid lands and climates over the world. This cactus plant was originated in America and then spread to other regions such as Europe, Africa, and Mediterranean countries. It is mainly cultivated for its seed, edible fruit (prickly pear), and cladodes. The nutritional and health benefits of the *Opuntia* genus are provided by various compounds such as phenolic compounds, pigments, polysaccharides, mucilage, dietary fibre, vitamins (B<sub>1</sub>, B<sub>2</sub>, A, and C), and minerals including magnesium, iron, calcium, potassium, and phosphorus. Owing to several health benefits, including prevention of diabetes, cancer, cardiovascular disease, and inhibition of inflammation, the number of studies focusing on developing novel foods and bioactive compounds by using different parts of this plant has increased recently. Several attempts have been made to integrate this plant into other foods, including bread, cake, pasta, gluten-free products, extrudates, cereal bars, juices, and meat products to improve their nutritional quality. In particular, *Opuntia* has gained importance as an excellent food source as desertification areas have increased and water resources have decreased globally. This chapter will discuss the recent studies dealing with the use of *Opuntia* spp. for edible purposes and the development of appropriate processing techniques to incorporate various parts of this valuable plant into other food matrices.

**Keywords** Edible · Cactus · Prickly pear · Bread · Cake · Pasta

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## Abbreviations

ABTS	2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
DIC	Instant controlled pressure drop (Détente instantanée contrôlée)
DPPH	2,2-diphenyl-1-picrylhydrazyl
GAE	Gallic acid equivalent
HHP	High hydrostatic pressure ultrasound treatment
PEF	Pulsed electrical field
QE	Catechin equivalent

## 1 Introduction

Cactus, which has many uses, poses an enormous potential to be the food of the future. *Opuntia* plant with approximately 300 species is a genus of the *Cactaceae* (cactus) family. Being native to North America (Mexico), the genus spread widely worldwide and grows in arid and semi-arid areas, particularly in African, European, and Mediterranean countries (Russell & Felker, 1987; Yahia & Sáenz, 2011). Owing to its high adaptation to harsh environmental conditions such as hot/cold climates, sun irradiation, and drought (Nobel & Bobich, 2002; Feugang, 2006), *Opuntia* spp. considered as a promising food crop.

When the extent of global desertification is taken into account, the incorporation of drought-resistant cactus plants into different food systems is indeed critical. The low-cost requirement in its cultivation and wide range of potential use areas make cactus plant a sustainable crop for food production purposes. Since this genus contains many attractive and valuable compounds, its use may contribute to malnutrition and increase population health (FAO, 2013; Reis et al., 2018). Therefore, the crop is worthy of attention in terms of nutritional and health benefits.

Morphologically *Opuntia* plant comprises the root, vegetable part (cladodes, pads, or stems), fruit (peel, pulp, seeds), and flower, while fruit and vegetable parts are mostly used for edible purposes. *Opuntia* fruits are also called “prickly pear”, “cactus pear” or “tuna” in various cultivation regions. The most cultivated species as food and natural additive sources are *O. ficus-indica*, *O. robusta*, *O. streptocantha*, *O. amyclaea*, *O. megacantha*, *O. hyptiacantha*, *O. rastera*, *O. engelmannii*, *O. phaeacantha*, *O. dillenii cactus* and *O. lindheimeri* (Stintzing & Carle, 2005; Yahia & Sáenz, 2011; Moussa-Ayoub et al., 2016). Chemical composition, bioactive compounds, and hence nutraceutical potential of the *Opuntia* spp. depend on several factors, such as region, variety, cultivation practices, climate conditions, plant age, and ripening stage (Rodriguez-Felix & Cantwell, 1988; Muñoz de Chávez et al., 1995; Reis et al., 2018; Al Juhaimi et al., 2020).

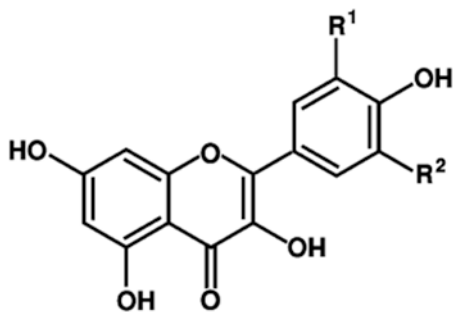
It is widely evidenced that fruits and vegetables have remedial effects against numerous diseases through their antioxidant, anti-inflammatory (Aiello et al., 2018; Ammar et al., 2018), antiulcerogenic (Alimi et al., 2011), anti-cancer (Zou et al.,

2005), cholesterol-reducing (Attanzio et al., 2019), immune-protective (Smida et al., 2017), diuretic, anti-stress, anti-diabetic, antimicrobial, and hypoglycemic (El-Mostafa et al., 2014; Aragona et al., 2018) activities.

The benefits of *Opuntia* fruits and stems in terms of nutrition and health have been attributed to assorted bioactive components, including pigments (mainly betalain and carotenoids), vitamin C and vitamin E (tocopherols) (Ramadan & Mörsel, 2003; Bourhia et al., 2020), and phenolic compounds including flavonoids (quercetin, isorhamnetin, kaempferol, pyrogallol, catechol, ferulic acid, quinic acid, rutin, hyperoside,) (Moussa-Ayoub et al., 2011; Ammar et al., 2018; El-Shahat et al., 2019). Mucilage (Du Toit et al., 2019) and fibre (Guevara-Arauz et al., 2015; Arias-Rico et al., 2020) are also prominent compounds. Prickly pears (as fruit) predominantly consist of quercetin, isorhamnetin, and kaempferol flavonoids (Fig. 46.1). Besides, *Opuntia* spp. has many other constituents having nutritional importance. Abundant fatty acids are linoleic, linolenic, palmitic, and oleic acids, some of which are essential for the human diet from nutritional and health perspectives (El-Mostafa et al., 2014; Al Juhaimi et al., 2020). Calcium, potassium, magnesium, and phosphorus are the main minerals required for the body's regulatory system. These elements and low sodium content are crucial for human health and take part in diverse body functions, including embracing electrolyte balance, nerve transmission, muscle contraction, bone growth, and disease prevention (Missaoui et al., 2020). Mineral richness can make *Opuntia* spp. a good supplement for commercial food, while its plenty of essential amino acid content can be utilized to enrich foods with natural origin ingredients (Feugang, 2006; Arba, 2020).

In terms of nutritional value, prickly pear is similar to other fruits, and even its soluble solid content (more than 16%) (Saenz, 2000) is higher than various fruits such as apple, citrus, and peach (Shao et al., 2011; Wang & Xie, 2014; Guo et al., 2016). Its high sugar content (93%, mainly glucose and fructose) (Farag et al., 2017) and low water activity provide high energy and enable the use of preservation

**Fig. 46.1** Structures of flavonoids (quercetin, kaempferol, and isorhamnetin) in cactus pear fruit (Adopted from Kuti (2004) with permission)



Quercetin ( $R^1 = \text{OH}$ ;  $R^2 = \text{H}$ )

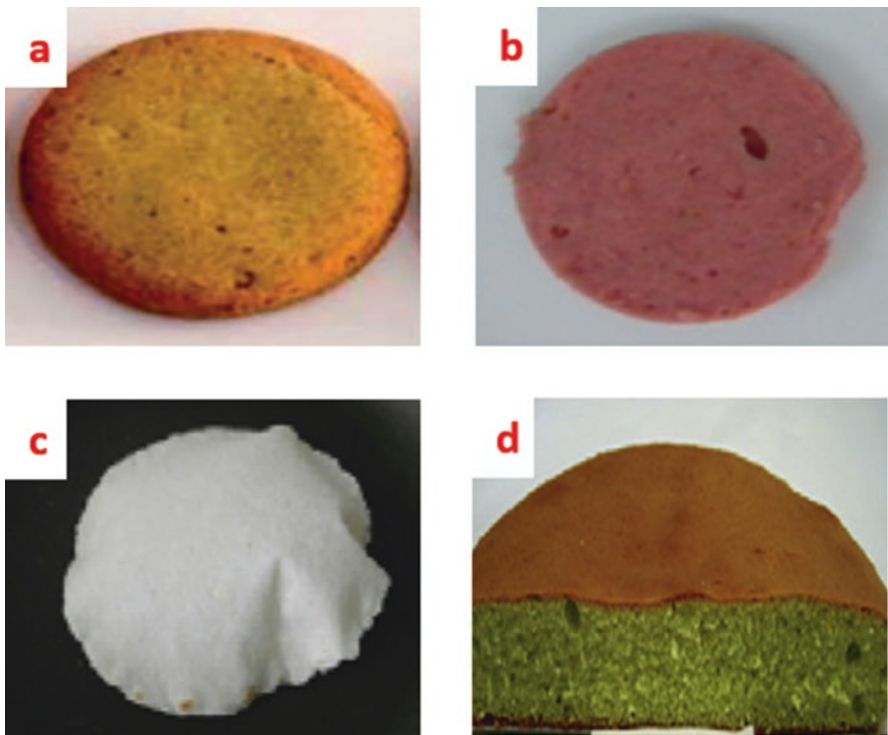
Kaempferol ( $R^1 = R^2 = \text{H}$ )

Isorhamnetin ( $R^1 = \text{OCH}_3$ ;  $R^2 = \text{H}$ )

techniques like concentration or dehydration (Saenz, 2000). Free amino acids of *O. ficus-indica* (L.) Mill. cover all essential amino acids. High levels of proline, taurine, glutamine, and serine were detected as primary amino acids. Some of them have structural, blood pressure regulative, and cell-protective functions (Stintzing et al., 2001; Stintzing & Carle, 2005; Aruwa et al., 2018). Organic acids (citric, malic, fumaric, and oxalic acid) contribute to the acidity and flavour of the fruits (Arias-Rico et al., 2020).

Besides pulp and peels (El Kossori et al., 1998), the fruit seeds are also nutritious fractions with 16.6% protein, 49.6% dietary fibre, and 17.2% lipid contents as well as the high amount of minerals, limiting (lysine), and sulphuric amino acids (methionine + cysteine) (Sawaya et al., 1983a). Considerable amounts of neutral lipids (87% of total lipids), linoleic, palmitic and oleic acids,  $\gamma$ -tocopherol (Ramadan & Mörsel, 2003), and phenolic acids, high antioxidant and antimicrobial activities (Chahdoura et al., 2015; Ramírez-Moreno et al., 2017) are the characteristics that endorse *Opuntia* spp. seeds in novel functional food applications.

These edible parts have various utilization potential in food systems due to differences among cladodes, fruits, pulps, and seeds depending on their



**Fig. 46.2** *Opuntia* spp. incorporated food products (a): cookie, (b): salami, (c): tortilla, and (d): cake (Adopted from Msaddak et al. (2015), Kharrat et al. (2018), Ramírez-Moreno et al. (2015), and Kim et al. (2012), respectively, with permission)

morphological, nutritional, dietary fibre, and mineral profiles. The chemical composition, nutritional, bioactive, and health-promoting properties of *Opuntia* spp. are incentives for its food area applications. A variety of products can be developed by processing or incorporating the fruits and cladodes into other matrices (Fig. 46.2). This chapter focuses on some of the potential uses of *Opuntia* spp. for edible purposes and valorization of its by-products in the food industry.

## 2 Potential Uses of *Opuntia* Spp. in Food and Alcoholic Beverages Industry

Various processes have been applied to produce different food products from fruits and cladodes of *Opuntia* spp. Table 46.1 summarizes some studies dealing with the utilization of cactus plants for food purposes.

### 2.1 Fresh-Cut Consumption

*Opuntia* spp. has been consumed as food and medicine since ancient times. They have been livelihood to rural populations in the arid areas of the world (FAO, 2013). Both fruits and vegetables are usually eaten fresh or further processed into various foodstuffs (i.e., juice, jam, edible film, pickle) and dietary supplements (Saenz, 2000; Moßhammer et al., 2006; Allegra et al., 2016; Wu, 2019) using different technologies and practices.

Cactus pear is prevalently cultivated for its tasty, flesh and colourful fruits, while cladodes (locally called nopales/nopals in Mexico) are usually consumed as vegetables. Young cladodes of 3–4 weeks are tender in structure and served as vegetables with meals. Protein and water contents are higher in young cladodes, whereas fibre content increases with the plant's age. The flours of prickly pear fruits and cladodes can be obtained when they slightly are lignified at 2–3 years (FAO, 2013). To consume as a vegetable, cladodes are washed, and their spines and glochids are removed. After peeling, the cladodes are sliced or cut into small cubes, then they can be eaten directly or boiled/cooked (Stintzing & Carle, 2005).

The sweetish acidic taste of cactus fruit results from high sugar and acid contents (mainly ascorbic and citric acids). Mostly they are consumed fresh, besides they are also put into use of various conventional and industrial uses (salads, sauces, desserts, alcoholic beverages, baked products, etc.). Though it varies according to the region and environmental factors, prickly fruit is generally susceptible to microbiological infection because of its high pH values (6.1–7.1), low acidity, and high soluble solid contents (Pimienta-Barrios, 1994; Arba, 2020). Thus, minimal processing techniques such as peeling, spine removing, slicing, and chopping bring about safety challenges due to microbial risk. Fresh cut processing of fruits requires

**Table 46.1** An overview of the utilization opportunities of *Opuntia* spp. cultivars in food industry

Food and by-products	<i>Opuntia</i> specie	Plant part	Used method	Reference
Pasta	<i>Opuntia ficus-indica</i>	Cladode	Fortification, 3%	Aiello et al. (2018)
	<i>Opuntia ficus indica</i> (L.) Mill.	Fruit	Fortification, 0–15%	Oniszczuk et al. (2020)
Bread	<i>Opuntia ficus-indica</i>	Seed	Fortification, 0–10%	Ali et al. (2020)
	<i>Opuntia ficus-indica</i>	Peel	Fortification, 1–2%	Anwar and Sallam (2016)
	<i>Opuntia ficus-indica</i>	Cladode	Fortification, 10–30%	Attanzio et al. (2019)
	<i>Opuntia ficus-indica</i>	Cladode	Replacing water	Liguori et al. (2020)
Edible coated-fig Edible coated- strawberry	<i>Opuntia ficus-indica</i>	Mucilage	With a mucilage solution	Allegra et al. (2017)
	<i>Opuntia ficus-indica</i>	Mucilage	With a mucilage solution	Del-Valle et al. (2005)
Edible film	<i>Opuntia ficus-indica</i>	Mucilage	Extraction, film-forming	Gheribi et al. (2018)
Snacks	<i>Opuntia ficus-indica</i>	Fruit	Fortification, 0–10% and extrusion	Moussa-Ayoub et al. (2015)
	<i>Opuntia dillenii</i>	Seed	Fortification, 0–20% and extrusion	Rayan et al. (2018)
	<i>Opuntia ficus-indica</i>	Cladode	Fortification, 0–10%, and mixing	Anchondo-Trejo et al. (2020)
Cake	<i>Opuntia ficus-indica</i>	Cladode	Fortification, 5–20%	Ayadi et al. (2009)
	<i>Opuntia humifusa</i>	Cladode/ fruit	Fortification, 0–9%	Kim et al. (2012)
	<i>Opuntia ficus-indica</i>	Cladode	Fortification, 0–10%	El-Safy (2013)
	<i>Opuntia macrorhiza</i> Engelm.	Cladode/ seed	Fortification, 15%, and 5%	Chahdoura et al. (2018)
Margarine	<i>Opuntia ficus-indica</i>	Peel	Incorporation, 50–150 ppm	Chougui et al. (2015)
Biscuit & cookie	<i>Opuntia ficus-indica</i>	Cladode	Fortification, 0–50%	De Wit et al. (2015)
	<i>Opuntia indica</i> & <i>Opuntia robusta</i>	Peel	Substitution, 7.5%	El-Shahat et al. (2019)
	<i>Opuntia ficus-indica</i>			
Mayonnaise	<i>Opuntia ficus-indica</i> & <i>Opuntia robusta</i>	Mucilage	Incorporation as replacer	Du Toit et al. (2019)
Yogurt colored	<i>Opuntia ficus-indica</i>	Fruit/ cladode	Encapsulation and coloring	Carmona et al. (2021)
Yogurt	<i>Opuntia ficus-indica</i>	Peel/ mucilage	Fortification & fermentation	Hernández-Carranza et al. (2019)

(continued)

**Table 46.1** (continued)

Food and by-products	<i>Opuntia</i> specie	Plant part	Used method	Reference
Juice	<i>Opuntia ficus-indica</i> <i>Opuntia dillenii</i> <i>Opuntia dillenii</i> Haw.	Fruit Fruit Fruit	Pasteurization/ storage PEF/HHP Pasteurization/ storage	Mata et al. (2016) Moussa-Ayoub et al. (2017) Bassama et al. (2021)
Biofunctional alcoholic beverage	<i>Opuntia ficus-indica</i> Mill.	Fruit	Alcoholic fermentation	Karabagias et al. (2020)
Probiotic beverage	<i>Opuntia</i> spp.	Fruit pulp	Fortification and fermentation	El-Sayed and Ramadan (2020)
Bakers' yeast	<i>Opuntia ficus-indica</i>	Fruit/peel	Inoculation and fermentation	Diboune et al. (2019)
Sausage	<i>Opuntia humifusa</i> f. <i>jeollaensis</i>	Fruit	Emulsification	Jeong and Han (2019)
Salami	<i>Opuntia stricta</i>	Fruit	Incorporation, 1–2,5%	Kharrat et al. (2018)
Sliced beef	<i>Opuntia ficus indica</i>	Fruit	Dipping and coating	Palmeri et al. (2018)
Dessert	<i>Opuntia elatior</i> Mill	Fruit	Juice extraction	Joshi et al. (2020)
Gummy candies	<i>Opuntia ficus-indica</i>	Fruit	Incorporation	Otálora et al. (2019)
Seed oil	<i>Opuntia albicarpa</i> & <i>Opuntia ficus indica</i>	Seed	Solvent extraction	Ramírez-Moreno et al. (2017)
Vinegar	<i>Opuntia ficus-indica</i>	Fruit	Fermentation	Prieto et al. (2009)
Wine	<i>Opuntia ficus-indica</i>	Fruit	Fermentation	Tsegay and Lemma (2020)

hygienic conditions and low temperatures, including post-harvest storage (suggested <10 °C) (Yahia & Sáenz, 2011).

Different packaging methods have been examined to extend the shelf life of minimally processed fruits. Prevention of microbial spoilage (particularly by yeasts and mesophilic bacteria) and the maintenance of nutritional and quality parameters are aimed at using suitable packaging techniques. In a relevant study, compared to the atmosphere (air), the modified atmosphere (65% N<sub>2</sub>, 30% CO<sub>2</sub>, 5% O<sub>2</sub>) strongly inhibited the growth of psychotropic bacteria and prolonged the cactus pear fruit's microbiological shelf life at low storage temperature (4 °C). Moreover, modified atmosphere packaging led to a more homogeneous bacterial population (Corbo et al., 2004). Generally, vitamin C decreases with storage depending on maturity, genotype, in-package gas concentration, and storage conditions. In minimally processed cactus pear fruits wrapped with heat-shrunk polyolefin films, (10%) loss of ascorbic acid occurred after 11 days at 4 °C (Piga et al., 2000). A more recent study,

shelf life of 8 days was provided by vacuum-applied polyolefin films (Palma et al., 2018). Furthermore, coating cactus pear with pomfresh (a mixture of organic acids and antioxidant components) (Palma et al., 2015) and alginate (Del Nobile et al., 2009) improved the shelf life. On the other hand, chitosan coating with 1% acetic acid was successful in inhibiting microbial growth. However, this application caused weight loss and softening (Ochoa-Velasco & Guerrero-Beltrán, 2014). Lately, the integrated use of  $\text{CaCl}_2$  dipping treatment, packaging with low-density polyethylene sheet, and storage under-ventilated evaporative cooler methods were recommended to increase retention of weight and sugar content, reduce decay loss, and prolong the shelf life of cactus pear fruits (Shumye Adilu et al., 2020).

## 2.2 Dehydrated Products, Jams, Purees, and Pulps

Dehydration is an ancient preservation method. Dehydrated products have a longer shelf life and are rich in dry matter, carbohydrates, and fibre. Mostly peels and seeds are separated as waste prior to industrial pulp and jam manufacturing. A pulp thus obtained (pH of 5.64, 13.47 °Brix) was shown as a good source of nutrients; vitamin C ( $243.4 \text{ mg L}^{-1}$ ), potassium ( $1180 \text{ mg L}^{-1}$ ), calcium ( $440.6 \text{ mg L}^{-1}$ ), and bioactive substances, for instance, phenolic compounds ( $120 \text{ mg L}^{-1}$ ), betalains ( $51.8 \text{ mg L}^{-1}$ ) and  $\beta$ -carotene ( $503.3 \text{ } \mu\text{g L}^{-1}$ ) (Lamia et al., 2018). In a pilot-scale production, the obtained prickly pear pulp (14.2% Brix) was characterized by high potassium, moderate calcium, and magnesium contents along with low amounts of vitamin A, iron, and sodium. A preliminary blanching of fruits did not cause any difference in the final sensory acceptance of the jams prepared with prickly pear pulp, date pulp, sugar, and various flavours (Sawaya et al., 1983b). Jam and marmalades are commonly consumed in many countries. Jams made with cactus cladodes, sugar, and citric acid in the proportion of 1:0.6:0.01 showed good sensory properties and microbiological stability (Badillo, 1987). In terms of sensory acceptability, no differences were found between cactus pear jams prepared with and without added lemon juice and peel (Vignoni et al., 1997). Also, panelists assigned very close scores to cactus fruit jams prepared with different sugar levels (70, 80, 90%), where all had high acceptance rates (Jotangiya & Samani, 2017). A low-calorie marmalade that exhibited similar sensory characteristics to standard marmalade containing sucrose sugar was produced using *O. ficus-indica* cladodes and low caloric sweeteners such as sugar alcohols (Leopoldo et al., 2012). The carotenoid contents of marmalades, particularly  $\beta$ -carotene and lutein, were higher than the fresh cladodes. The authors explained this finding by the thermal (at 90 °C for 35 min) rupture of the complexes like carotenoid-protein which may trigger the release of more carotenoids from the matrix. On the other hand, a decrease in the  $\alpha$ -cryptoxanthin content was observed since it was more susceptible to hydrolysis in the oxidation processes, as was suggested by the authors. Compared to the fresh nopal, higher

antioxidant activities of marmalades were attributed to the thermal effect increasing the extractability of antioxidant bioactive like carotenoids.

A natural red cactus pear fruit puree as a microbiologically safe product in coliforms, lactic acid bacteria, *E. coli*, and aerobic microorganisms besides yeasts and fungi was produced in the early decades of the twentieth-century (Thomas, 1998). Concentrated puree (65 °Brix) manufactured through vacuum-dehydrators was suggested as a flavouring agent for ice creams, desserts, and pastries. Cactus pear fruit puree that was slightly concentrated (37%) and modified in acidity (pH =4, with citric acid) exhibited similar color, aroma, and flavour to that of its natural pulp; evidencing that it could be a convenient constituent for the confectionery industry as a semi-processed product (Barbagallo et al., 1998).

Grinding dehydrated *Opuntia*'s cladodes mostly yields a powder with a slight brown color used as a functional ingredient to functionalize foodstuff and formulate nutraceutical products. Solar energy can be used as an economical and environmentally friendly technology, especially in semi-arid areas where cactus grows widely. Recently, using a solar air heating system combined with a ventilation to dehydrate *Opuntia ficus-indica* cladodes ensured chips and powders with preserved natural green-yellow color (Ciriminna et al., 2019). Additionally, the optimized osmotic dehydration pre-treatment coupled with the hot air drying process reduced more moisture content and preserved the quality properties of nopals compared to the application of the hot air drying process alone (Rodriguez et al., 2019). Using polyethylene glycol as a dehydration agent at increased concentrations; provided better rehydration capability and sensory properties than hot air or freeze-drying treatments, besides retaining the color (Yu et al., 2010). For direct consumption, cactus pear fruit pieces (1 gr) were obtained through environmental-friendly solar-air dryers (50–60 °C) (Lahsasni et al., 2004). Cactus fruit sheets made of cactus pears: quince pulp (75:25) without preservatives were developed by use of forced air tunnel dehydrator (57–60 °C, 6–8 h) (Sepúlveda et al., 2000). Adding 2–3% sucrose to the pulps in an air oven (60 °C/44 h) provided well-accepted edible cactus sheets (El-Samahy et al., 2007). The dehydration rate decreased by increasing the pulp layer's thickness (from 5 to 15 mm) and was faster at 70 °C. Process conditions define quality and rheological properties. In terms of protecting bioactive compounds, the convective drying applied at 45 °C with 3 m s<sup>-1</sup> of airflow rate was detected as the best condition during dehydration of *Opuntia ficus-indica* cladodes (Medina-Torres et al., 2011). Ascorbic acid was the most severely affected bioactive compound. Only 25% of flavonols remained, but more than 60% of total phenols and more than 80% of total flavonoids were protected. Samples dried at 45 °C showed Non-Newtonian shear-thinning behaviour, while shear-thickening observed at 65 °C was associated with thermal degradation. These studies give notable data regarding the effect of processing (mixing, flow processing) on the texture of rehydrated samples. Small-scale production of such dehydrated products is relatively easy and involves low-cost technologies.



### 2.3 Juices and Derivatives

*Opuntia* spp. has been utilized for their delicious juices with attractive colours. Consumption of cactus pear juices may present substantial health benefits using various bioactive molecules. Lamia et al. (2018) formulated nectars with 35–45% *Opuntia ficus-indica* fruit pulp, 11% sucrose, and 0.3% citric acid, and resulted in 14–15 °Brix values after pasteurization at 80 °C/15 min. The stability and acceptability of these natural beverages (no added synthetic preservatives/colorings/sweeteners) after 1 month was confirmed through stability and panel tests. Nectar of 45% pulp provided 1112 mg L<sup>-1</sup> of potassium, 362.8 µg L<sup>-1</sup> of β-carotene, 231 mg L<sup>-1</sup> of vitamin C, and 5.7 mg L<sup>-1</sup> GAE of total phenolics. A bottle of 250 mL was enough to cover the approximate nutritional requirement of a child (Lamia et al., 2018). A total of 44 bioactive compounds were detected in *Opuntia* spp. juices, where 32 were characterized by liquid chromatography and tandem mass spectrometry (Mata et al., 2016). The major ones were the isorhamnetin and quercetin aglycones from flavonoids; the caffeic and ferulic acid derivatives from hydroxycinnamic acids. Among betaxanthins, indicaxanthin was the main yellow pigment, whereas betanin and isobetanin represented the major red betacyanins, acting as antioxidants. Phenolic acid, phenyl pyruvic acid, and piscidic acid derivatives were also detected in the extracts. These data indicate cactus juice as a good source of functional bioactive compounds. The phenolic and antioxidant content of juices is affected by their cultivar. This was demonstrated by a study where total phenolic contents of red cactus juices (1152.9 and 1065.1 mg GAE/100 mL) were higher than that of yellow cactus juices (786 and 667 mg GAE/100 mL) (Abdel-Hameed et al., 2014).

Because of their high pH values, cactus pear juices require procedures to stabilize and preserve their microbiological quality without (or with minimal) damage to color, taste, and nutrients (Barba et al., 2020). Osmotic membrane distillation can be used to obtain semi-concentrated juices. Recently, cactus pear juice concentration was increased by around 50% with this technology (Terki et al., 2018). Increasing process time (from 5 h to 18 h) and temperature (from 20 °C to 35 °C), refreshing stripping solution and using PTFE membrane instead of PP enabled to get higher final juice concentrate and water permeate flux. After the membrane distillation process, juice displayed higher phenolic and flavonoid contents and antioxidant activity, owing to increased bioactive compounds.

The stability against detrimental microorganisms could be achieved by producing concentrated juices (>63 °Brix), which have low water activity. However, this time, undesirable color and aroma features may come up due to the concentration procedures (mostly by evaporation) (Saenz, 2000). Thermal treatments may inhibit pathogenic microorganisms and prevent spoilage; however, some negative impacts on the physical, sensorial, and nutritional quality of the foods may occur. An increase or decrease of phenolic and antioxidant potentials in plant-based beverages may be possible, depending on product structure and severity of the thermal processes (Barba et al., 2017; Taşkın & Aksoylu Özbek, 2020). In cactus pear juice

(*Opuntia dillenii* Haw.), betacyanins' thermal degradation behaviour (initial content was 0.76 g/kg) was described over a temperature range between 60 to 90 °C. The maximum betacyanin loss was observed as 10% when temperatures were above 80 °C. *Enterococcus faecalis* was taken as the reference microorganism in terms of food safety, and for an efficient inhibition, the satisfying time/temperature combinations were suggested as 100 °C/0.6 min or 62 °C/180 min. During storage of pasteurized juice (70 °C, 30 min), degradation kinetics of betacyanins increased positively with temperature (from 4 °C to 45 °C). Degradation was accompanied by the formation of brownish color which was also demonstrated by increased browning index (Bassama et al., 2021).

High hydrostatic pressure (HHP), ultrasound treatment, and pulsed electrical field (PEF) are emerging technologies developed against quality impairments in juice processing. HHP, as a non-thermal treatment, retained and even enhanced the bioactivity of prickly pear beverages compared to fresh or heat-treated ones (Jiménez-Aguilar et al., 2015). *Opuntia* beverages prepared with 10% peel and 90% pulp were subjected to HHP treatment (400 or 550 MPa, ambient temperature, 16 min). While total phenolic content (16–35%) and antioxidant capacity (8–17%) significantly increased in HPP-treated samples (550 MPa/t  $\geq$  2 min), kaempferol, and isorhamnetin amounts maintained, and vitamin C decreased in some quantity (3–15%). In contrast, total phenolics, flavonoids, betalains, and antioxidant capacity heavily decreased in thermally sterilized samples (131 °C, 2 s).

PEF is another growing technology that was preferred for juice preservation in the market. Electric field pulses impress the permeability of biological membranes and modify their permeabilization depending on the desired process effect. With regards to conservation, a high strength of electric field (>20 kV/cm) is adequate to inactivate vegetative microorganisms using irreversible permeabilization in the cell membrane (Sánchez-Vega et al., 2015; Barba et al., 2015). During *Opuntia dillenii* juice production, PEF (35 kV/cm, 85 kJ/kg) or HHP (600 Mpa, 35 °C, 10 min) provided a microbiological reduction similar to that of conventional thermal heating (95 °C, 3 min). Both PEF and HHP exerted a mild impact on rheological properties. When compared to thermal treatment, they provided better protection on sensitive bioactive compounds along with higher antioxidant activities (Moussa-Ayoub et al., 2017). Processing fruits rather than consuming them fresh can have beneficial consequences. The juices' flavonol content was higher than that of untreated fresh juices, which indicated the advantage of processing. The increased yield was attributed to improved cell disintegration and the more release of bioactive compounds, as was observed in a previous PEF study of the same cactus species (Moussa-Ayoub et al., 2016). Moreover, using PEF technology in combination with other applications like pH reduction and the use of antimicrobials (potassium sorbate, sodium benzoate) can be a good alternative for stabilizing the prickly pear beverages during storage periods (21 days/25 °C) (García-García et al., 2015).

Recently, ultrasound has been used as another non-conventional conservation method for prickly pear juice manufacturing. Performing the treatment longer than 5 min enabled an inactivation of target pathogens (*E. coli*) without any change in soluble solids' pH and content (Cruz-Cansino et al., 2016). Protected phenolic and

antioxidant compounds (except sensitive ascorbic acid loss by 20%), besides reducing indicative bacteria population, were revealed after 15 min treatment periods (Cruz-Cansino et al., 2013). The thermo-ultrasound process at 50 °C provided bacterial inhibition and physical stability during 21 days of storage; however, it could not prevent the viscosity decrease, browning in color, and reduce betaxhantins (Cruz-Cansino et al., 2015). Thus, color, taste, and nutraceutical quality attributes should also be considered while determining the juice production process conditions.

## 2.4 Fermentation-Derived Beverages and Other Products

Existence of bioactive nutrients and functional compounds in *Opuntia* spp. encourages developing different food products with nutritional and health-promoting properties (El-Mostafa et al., 2014; Aragona et al., 2018; Missaoui et al., 2020). The nutritional value of cactus plants can be enhanced through fermentation. Utilizing this process, the nutrients and bioactive constituents can be transferred into the daily diet. Ingestion of some synthesized microbial metabolites can contribute to health. Mainly, lactic acid fermentation is an alternative way to extend shelf life and promoting nutritional and functional properties of food products. Lactic acid and antimicrobial agents produced by lactic acid bacteria inhibit pathogenic and spoilage microorganisms and increase the food's durability while creating different aromas and flavours (Hashemi et al., 2017; Verón et al., 2019; Koubaa et al., 2019).

Various fermented beverages of different characteristics can be produced *via* lactic acid fermentation of both fruit and cladodes. In general, fermentation by lactic acid bacteria (usually at 28–37 °C for 48 h or until a certain pH degree) increases the amount of the lactic acid and decreases the initial pH value due to consumption of sugar source, which is then the adequate condition for the *Lactobacillus* strains to grow (Panda et al., 2017; Verón et al., 2019). Along the process, new organic compounds like organic acids, aromatic carboxylic acids, alkenes, hydroxyl/nitro groups, and alcohols are produced, plus phenolic substances are protected, even increased (Randazzo et al., 2016; Panda et al., 2017). Fermentation of prickly pear fruit with *Lactobacillus* spp. (*L. rhamnosus* LS, *L. bulgaricus*, and *L. brevis*) was performed after pasteurization (80 °C/10 min) of its aqueous solution. Additions of 3% lactic acid bacteria, 5% fructose syrup, 0.2% yeast extract, and 0.05% CaCO<sub>3</sub> before incubation enabled get a beverage in which viable lactic acid bacteria cells and the color features were well maintained during 3 weeks at 4 °C (Son & Lee, 2004). Prieto et al. (2009) produced balsamic vinegar from concentrated (30 °Brix) orange, purple, and green colored *Opuntia* cactus pear juices fermented with *Saccharomyces cerevisiae* yeast for 3 to 5 days to reach 4–5 °GL alcohol degree. After yeast fermentation, the vinegar was acetified with acetic bacteria. In terms of sensorial quality, acidity and sweetness, the best vinegar was obtained from purple cactus pear.

*Opuntia* spp. has also been utilized in order to produce or develop probiotic food products. Both fruit and cladode pulps are adequate for the growth of starter

cultures, lactic acid bacteria, and fermentative or probiotic strains (Barba et al., 2020). In a recent study, a fermented non-dairy kefir beverage was obtained by using water-kefir microorganisms. Prickly pear (*Opuntia ficus-indica*) kefir exerted the highest yeast and lactic acid bacteria growth along with the maximum lactic acid content (1 g/L), among its counterparts prepared by apple, grape, quince, kiwi, and pomegranate. A significant increase of volatiles, acids, and alcohols was obtained after fermentation. Production of aldehydes and ketones were more pronounced in quince, prickly pear, and kiwi kefir. Lighter color was observed after fermentation. However, during overall quality comparison, cactus kefir was less preferred than apple and grape (Randazzo et al., 2016). The innovative vegetable-based milk beverages are of attention in response to discomforts related to lactose intolerance. *Opuntia* spp. was also incorporated into non-dairy probiotic functional food products to balance the nutritional value. Preliminary pasteurized rice milk was fortified with cactus pear (20% fruit pulp) and fermented with 5% bacterial culture (*L. acidophilus*, *S. thermophiles*, and *Bifidobacterium bifidum*). Fortification with fruit pulp significantly increased DPPH radical scavenging inhibition activity and phenolic content of fermented rice milk. At the end of fermentation, beverage fortified with *Opuntia* spp. received the highest sensorial acceptability score than beverages from *Physalis peruviana* fruit and rice milk (as control) (El-Sayed & Ramadan, 2020). Based on these results, it can be suggested to derive probiotic products improved in nutritional quality and bioactivity by using *Opuntia* components.

Fermentation by selected autochthonous lactic acid bacteria is quite often preferred as an alternative to spontaneous fermentation by lactic acid bacteria present in the microbiota. Autochthonous strains may be selected and adapted specifically for the food matrix to assure sufficient inhibition of undesirable microorganisms and the off-quality features. A prickly pear puree which was fermented with autochthonous lactic acid bacteria strains (*Leuc. mesenteroides*) had a longer shelf life. Fermentation generated a valuable alternative to its harvest surpluses of perishable fruit forms due to better color, sensorial, and health-promoting features (Di Cagno et al., 2016). Throughout the fermentation, glucose, fructose, and citric acid contents declined. The primary metabolites produced were lactic acid and acetic acid. Color parameters, sensory features, antimicrobial activity, and browning index were positively influenced by lactic acid fermentation. Preservation of betalains and vitamin C were recorded. Fermented puree with bacteria strains also inhibited the inflammatory status of Caco-2/TC7 cells in response to TNF- $\alpha$ , IL-1b, and IFN- $\gamma$  and improved the integrity of tight junctions. Fermented cactus products may serve as promising models for nutraceutical and functional foods to be developed.

Recently, another autochthonous strain, *Lactobacillus plantarum* S-811, has been used to ferment cactus (*Opuntia ficus-indica*) juice, and the fermentation effect on oxidation stress was evaluated (Verón et al., 2019). The antioxidant activity was preserved and even increased as contrary to the findings of Panda et al. (2017), who fermented cactus juice with *L. fermentum* and reported about a 30% decrease in antioxidant activity. Applied fermentation significantly strengthened the tolerance against oxidative stress induced by exposure to H<sub>2</sub>O<sub>2</sub>, thus assisting health-promoting properties. According to the results in obese mice, fermented cactus

juices revealed a potential for preventing obesity and related pathologies, such as insulin resistance and hyperglycemia.

Natural glycosides (such as isorhamnetin that is one of the major flavonoids in cactus cladodes) are supposed to convert into their aglycones to be more bioavailable (Manach et al., 2005). Therefore, it is the subject of interest to transfer glycosides into aglycones using either enzymes or microorganisms in many studies (Moussa-Ayoub et al., 2011; Li et al., 2016). In Korea, the eastern prickly pear *Opuntia humifusa* is known for its potential of bioactive and nutraceutical components. However, its mucilaginous nature obstructs its processing to juices or efficient extraction of valuable substances from the matrix. The fermentation treatment at 37 °C for 60 h with either  $\beta$ -glucosidase-active lactic acid bacterium (1% w/w) or cell wall-hydrolyzing enzymes (4 units/g cladode, particularly viscozyme) allowed breaking down mucilaginous barrier structure. This resulted in enhanced juice yield efficiency and developed bioactive properties. On average, juice yield increased by up to 48%, where total phenolics and antioxidant activity increased by more than 50%. Meanwhile, the amount of flavonoid glycosides decreased, which designated their conversion into aglycones. Besides the rise of flavonoid content by 4 times, the quantity of flavonoid glycosides declined, which designates their conversion into aglycones (Quines-Lagmay et al., 2020).

Alcoholic beverages like wine and brandy can also be produced from cactus fruits and juices-thereof (FAO, 2013; Tsegay et al., 2018). Fermentation temperature, pH, and inoculum size are prominent factors affecting wine quality (Sudheer Kumar et al., 2009; Lee & Chen, 2016; Tchabo et al., 2017). The response surface optimization approach can predict optimal fermentation conditions in prickly pear wine production with desired characteristics. Tsegay et al. (2018) revealed that there was a significant relationship ( $p < 0.05$ ) between the quality-based response parameters and fermentation process conditions (temperature, pH, and inoculum percentage) in the production. Temperature, pH, and yeast inoculum concentration were positively sufficient on total acidity and antioxidant substances. The optimum conditions were suggested as 30 °C fermentation temperature, 3.9 pH level, and 16% yeast (*Saccharomyces cerevisiae*) inoculum concentration to get *Opuntia* fruit wine with the comparable total acidity (12.3 g/L, as tartaric acid equivalent), moderately low alcohol content (9%, v/v), the total antioxidant capacity of 235 mg/L (ascorbic acid equivalent), and high sensory acceptance score (7.74) over the 9-point hedonic scale.

Various fruits can be blended and fermented to improve wines' final quality (Lee & Chen, 2016). Incorporating *Lantana camara* (flowering plant belonging to verbena family, *Verbenaceae*) fruit juice into the fermentation process has increased total phenol content (651.6 mg GAE/L) and overall sensorial acceptability of cactus fruit wine (Tsegay & Lemma, 2020). This contribution is possibly raised from additional bioactive compounds available in the fruit. Increasing yeast inoculum concentration increased the conversion of nonphenolic structure into phenolic compounds. Moreover, yeasts' hydrolytic enzymes can release soluble phenolic substances from fruit cell walls, thus enhancing phenolic content (Tchabo et al., 2017). Alcohol degree declined when the yeast concentration increased. This was

attributed to the growth and reproduction of yeast cells affected by initial nutritional status of the media. As explained by the authors; during fermentation process, metabolic reaction slows down because of ongoing nutrients consumption, and the release of further CO<sub>2</sub> suppresses the alcohol production.

More recently, alcoholic fermentation of prickly juice and its pulp was conducted using a blend of sugar, blossom honey, and *Saccharomyces cerevisiae* yeast. The fermentation provided a beverage with a fruity, floral honey-like aroma that originated from primary alcohols, ethyl esters, 3-hydroxy-2-butanone, benzaldehyde, and butanedioic acid ethyl ester, and phenolic volatiles. After alcoholic fermentation, the alcohol level was 23%. In vitro antioxidant activity (64%) and total phenolic content (7.6 mg GAE/mL) of fermented beverage were higher than its juice form (Karabagias et al., 2020). Producing bio-functional beverages by alcohol fermentation can be an excellent option to increase bioactivity and extend the shelf life of semi-perishable fruits like a cactus pear.

The cactus cladodes were also proven to transform into their functionally-improved forms by the fermentation process. Protein and essential amino acid contents of *Opuntia ficus-indica* cladode biomass were increased by fermentative metabolism of *C. utilize* and *K. marxianus* yeasts. Amino acid contents, in particular lysine, increased by around threefold. Although its amino acid profile was comparable to that of cereals, the lysine and sulphuric amino acid levels were below the FAO/WHO scoring pattern (Akanni et al., 2015). During *Opuntia* cladode, pulp fermentation (30 °C for 24 h), *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus rossiae*, and *Pediococcus pentosaceus* were the allochthonous starter strains exhibiting the highest growth (Filannino et al., 2016). Fermentation insured protection on carotenoids and vitamin C. Especially *L. plantarum* and *L. brevis* cultures magnified the pulps' immune-modulative and radical scavenging capability. Fermented cladode pulp showed higher inhibition of IL-8, TNF $\alpha$ , and prostaglandins PGE2 synthesis than control which was acidified without fermentation. This improvement most likely arises from kaempferol and isorhamnetin flavonoid derivatives measured in high concentrations.

Pickles and “tuna cheese (briefly, a kind of cheese made by boiling, concentrating, beating, and drying the pulp and juice) are other fermented-derived products from *Opuntia* spp. (Saenz, 2000). Pickling of cladodes gives them a slightly sour taste. Pickled and in brine derivatives of cactus parts are popular in some regions like Mexico. Mostly vinegar (1.8–2%), herbs, and spices are used for pickles. Brine may contain up to 2% sodium chloride for preservation aim (Saenz, 2000; FAO, 2013; Barba et al., 2020).

## 2.5 Flours, Bakeries, and Snacks

Today, there is a continuous development in the food industry to meet the growing consumer demand for nutritious, functional, low-calorie, low-fat, and healthy products. The current trend is to find new sources of natural and nutritionally valuable

**Table 46.2** Physical and chemical properties of some flours/powders of *Opuntia* spp.

Parameter	Cladodes	Fruit/Peel	Seeds
Source	<i>Opuntia ficus-indica</i> , <i>Opuntia ficus indica f.</i> <i>Amylocea</i> , <i>O. ficus indica f. inermis</i> , <i>Opuntia ficus indica (L.)</i> <i>Miller</i> , <i>Opuntia macrorhiza Engelm.</i> , <i>Opuntia boldinghii Britton et</i> <i>Rose</i>	<i>Opuntia ficus-indica</i> , <i>Opuntia ficus-indica L.</i> , <i>Opuntia xocconostle</i> , <i>Opuntia matudae</i>	<i>Opuntia</i> <i>ficus-indica</i> <i>Opuntia</i> <i>macrorhiza</i> <i>Engelm.</i> <i>Opuntia dillenii</i> <i>Opuntia albicarpa</i>
Moisture %	2.0–10.0	7.2–11.2	4.2–5.2
Ash %	16.7–28.9	8.7–14.6	1.6–5.9
Carbohydrates %	45.0–67.6	49.6–85.4	23.3–55.5
Crude protein %	7.2–13.2	3.3–8.3	10.0–14.6
Fat %	1.1–2.4	2.5–3.7	6.8–10.5
Dietary fibre %	41.5–55.1	30.8–64.1	50.8–54.2
Crude fibre %	6.0–9.6	6.4–7.7	18.23–52.8
pH	4.3–4.4	5.2 - 5.5	–
Total phenolics (mg GAE/100 g)	976–2485	348–2776	39.56–574
Flavonoids (mg CE/100 g)	–	15.8–32.5	102.7–595
β-Carotene (mg/100 g)	0.7–7.75	7.6–336.8	–
DPPH (%)	–	11.15–82.7	62.76
Water holding capacity (g/g)	7.9–14.44	1.8–6.0	23.69
Oil holding capacity (mL/g)	1.3–2.8	0.9–4.3	1.45
References	Anchondo-Trejo et al. (2020), Ayadi et al. (2009), Cornejo-Villegas et al. (2010), Chahdoura et al. (2018), El-Safy (2013), Moreno-Álvarez et al. (2009), López-Cervantes et al. (2011), (Msaddak et al. (2015) and Ramírez-Moreno et al. (2015)	Anwar and Sallam (2016), Arias-Rico et al. (2020), Bouazizi et al. (2020), Diaz-Vela et al. (2013), El-Shahat et al. (2019), Mahloko et al. (2019), Parafati et al. (2020) and Ruiz-Gutiérrez et al. (2017)	Ali et al. (2020), Chahdoura et al. (2018), Rayan et al. (2018), Reda and Atsbha (2019) and Sawaya et al. (1983a)

Results were given on a dry weight basis

ingredients, including agricultural by-products that have been traditionally undervalued. Moreover, the interest tended to transfer these food sources' superior attributes or their derivatives into new products and their quality.

Botanical parts from *Opuntia* have been studied for many different purposes. Due to their health-beneficial effects (Aragona et al., 2018; Ammar et al., 2018), techno-functional properties (Arias-Rico et al., 2020), and potential role in improving the nutritional profile of other matrices (Aruwa et al., 2018; Chahdoura et al., 2018; Du Toit et al., 2019; Diboune et al., 2019), flours (or extracts) of cactus fruits, cladodes, and seeds can be exploited in various food systems (Table 46.2). With this purpose, alternative methods have been established in different industries such as bakery, pasta, snack, and extrusion (Sarkar et al., 2011; Guevara-Arauz et al., 2015; Rayan et al., 2018; Liguori et al., 2020; Oniszczuk et al., 2020).

The role of natural sourced dietary fibre in the prevention against diseases like diabetes, obesity, and digestive discomforts has been well demonstrated (Wolfram & Ismail-Beigi, 2011; Chong et al., 2014). Cladode flours can be obtained from their matured forms (2–3 years), and in cladodes or stem the fibre content increases with aging (FAO, 2013). By adjusting drying temperature-time combinations, a flour with 43% dietary fibre (where 28.5% was insoluble) could be obtained (Saenz, 2000). Cladode flours may have various compositional and functional properties when dried over different temperatures. For instance, drying at 80 °C limited the water holding capacity and green color intensity whereas, better quality attributes were obtained at 60 °C. Meanwhile, tyrosine, proline, aspartic acid, and glutamic acid were the primary amino acids; palmitic acid (C16:0), linoleic acid (C18:2n6), and linolenic acid (C18:3n3) were the abundant fatty acids in flours after heat treatments (López-Cervantes et al., 2011).

Different strategies were developed to improve biological and functional values, such as incorporating cactus plant components into various food formulations. Cactus fruit peels are the ideal carbon sources for lactic acid bacteria. Fermented cactus pear (*Opuntia ficus-indica* L.) peel flours exerted higher carbohydrate and fibre content and better antioxidant activity than fermented pineapple peel flours (Diaz-Vela et al., 2013). The cladode flours (dietary fibre; 41.83 to 51.24%) were incorporated into wheat flours within the range of 5%-20% altered wheat dough properties. Dough tenacity, energy value, adhesion, stickiness, and hardness increased in line with flour fortification, while a decrease in elasticity (due to higher fibre content) and lightness was observed (Ayadi et al., 2009). However, in another study, a reduction in extensibility was reported besides enhanced softening degree and elasticity (El-Safy, 2013).

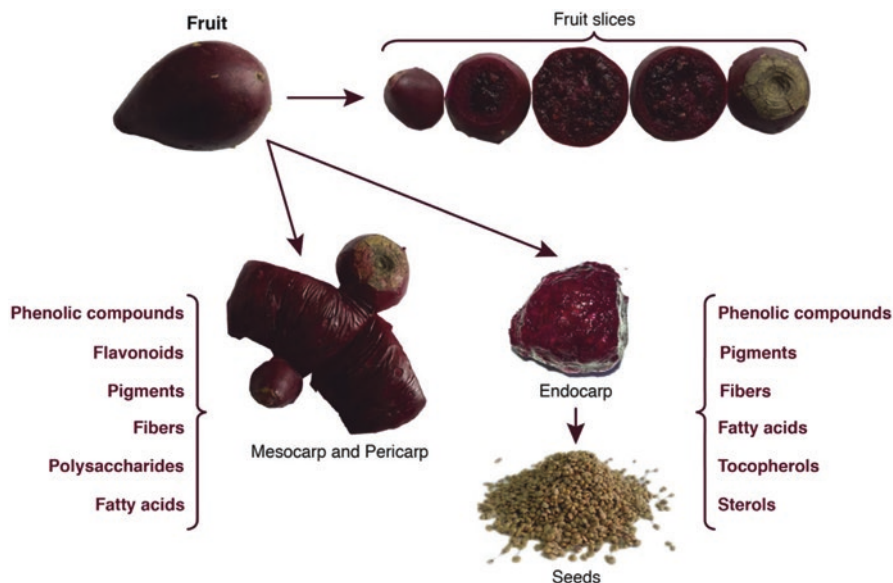
Fortification with varying proportions of ingredients will modify the flavour, color, and texture of the products. Incorporating *Opuntia* cladodes (such as *O. robusta*, *O. ficus indica*, and *O. humifusa*) up to certain levels into cake formulations was beneficial to technological and sensorial attributes. In general; as the substitution level increased, ash content, fibre content, swelling ability, specific gravity, the yield of dough, energy, and hardness increased, while elasticity, calorie level, carbohydrate, protein, and fat contents decreased (Ayadi et al., 2009; Kim et al., 2012; El-Safy, 2013; Chahdoura et al., 2018). It is expected that fortification of cakes with the highly nutritive, low calorie, and the bioactive cactus plant will promote health benefits. However, the substitution concentration is the primary factor to be optimized as the addition of cladode flours at high ratios may result in



undesired textural and sensorial attributes. Mostly, a partial substitution of (wheat) cake flour up to 10–15% levels provided a pleasant texture and well-accepted sensorial properties without detrimental effects to quality (Ayadi et al., 2009; El-Safy, 2013; Chahdoura et al., 2018). The stiffer, sticky, and less elastic texture obtained at high substitution ratios (more than 15%-25%) resulted in cakes of low volume and a more compact body. Surface breakdown with appeared cracks was observed. The increased tenacity and decreased elasticity might be due to rising water holding capacity and less gluten formation than control cakes. The rheological and textural alterations maybe because of dilution of gluten proteins, interactions between fibrous materials and gluten, or those between polysaccharides of cladodes and proteins of wheat flour (Ayadi et al., 2009; De Wit et al., 2015). In general, when the cladode powder level increased, the cake had denser green color, *L* (lightness) and *b* (yellowness) value decreased, and the crumb color became darker (Kim et al., 2012; El-Safy, 2013; De Wit et al., 2015).

Cactus fibers can also benefit from fat and sugar substitutes to increase swelling, oil, and water-binding capacity or control oxidative processes in food formulations (El-Safy, 2013; Anwar & Sallam, 2016; Arias-Rico et al., 2020). As a significant source of fibre (32.67%), pectin (14.2%), ascorbic acid (87.8%), and other bioactive components (phenolics, flavonoids, syringic acid, gallic acid), the prickly pear peel flour was integrated into pan bread production. Fortification (substitution levels of 1–2%) improved nutritional and baking quality (increased loaf and specific volume), reduced the staling (improved water retention) and extended its shelf-life (Anwar & Sallam, 2016). On the other side, in bread rolls formulated with cactus fibres, the best quality features (crumb volume, hardness, staling) and the highest sensorial preference scores were obtained with soluble fibres rather than its total or insoluble forms (Guevara-Arauz et al., 2015).

A diet including antioxidant-rich foods is a preventive way against many diseases. Partial replacement of wheat flour with cladode powder (from 0% to 10%) resulted in remarkable modifications on dough characteristics and the quality of bread made from the dough (Msaddak et al., 2017). At high substitution levels, extensibility and deformation energy was declined, showing that a high level of substitution negatively affects dough development. However, increased fortification level (to around 10%) increased the bread yield by 18.8%, possibly owing to high water retention enhanced by cladodes. Substitution level at 5% was found to be optimal for improving specific volume (1.50%), total phenolics (5.22 mg GAE/100 g), and the antioxidant potential (DPPH radical scavenging ability of 14.6%, the chelating activity of 33.8%) of bread. However, additional cladode powders resulted in decreased crust and crumb color parameters ( $L^*$ ,  $a^*$  and  $b^*$ ), as was expected. Incorporation levels up to 20% produced bread with higher dietary fibre (6.5%) and ash contents, and lower protein and fat contents. Achieved minimum protein amount above 13% was acceptable for a normal market bread. Moreover, the reduced-fat amount was regarded as beneficial for health. (Moreno-Álvarez et al., 2009). Increased water absorption capacity was in line with other studies associated with the increase of fibre (Ayadi et al., 2009; El-Safy, 2013). At high substitution levels (17–20%), decreased dough stability (an index for dough strength



**Fig. 46.3** Valuable compounds from various *Opuntia stricta* Haw. fruit parts (Adopted from Barba et al. (2017))

originates from gluten network) (Moreno-Álvarez et al., 2009), lower bread volume, firmer texture, and darker color (De Wit et al., 2015) are the significant consequences that may be ameliorated. It can be concluded that the optimum sensory results can be obtained in the 5–10% substitution range in the light of the reported studies.

Recently, revalorization of the by-products of *Opuntia* spp. has increased. In the industry, up to 45% of the (fresh) weight of fruits is separated as a discard (Bensadón et al., 2010). This is mainly composed of peels and seeds, which are good sources of antioxidants, vitamins, lipid, and fibers (Aruwa et al., 2018). The main part of *Opuntia* spp. the fruit is the edible pulp (flesh), and 15% of the total pulp weight is constituted by seeds. On the other hand, peel (mesocarp and pericarp) composes about 40% of the whole fruit weight (Barba et al., 2017, 2020) (Fig. 46.3). The seeds are tightly packed in a mucilaginous structure within the endocarp and offer various health benefits due to their composition rich in dietary fibre, phenolics, antioxidant and antimicrobial compounds (El Kossori et al., 1998; Ramírez-Moreno et al., 2017; Ali et al., 2020). As with cladode and stems, *Opuntia ficus-indica* fruit seed flour can be consumed *via* mixed with wheat flours up to a limited ratio. In formulated bread, roasted prickly pear seed flour with wheat flour raised dietary fibre and fat contents while reducing the energy and carbohydrate levels. Specific volume and bread volume was decreased, possibly because of the fibres interfering with the gluten network formation. Total phenolics and DPPH radical scavenging antioxidant activity increased with fortification concentration and reached the highest values at 6, 8, and 10%. However, more than 6% seed flour supplementation was

unacceptable in sensorial quality (Ali et al., 2020). However, the substitution of wheat flour only up to 15% was preferable as the sensory scores of traditional “Himbasha” bread decreased at other levels (Reda & Atspha, 2019). In a more recent study, added fresh *O. ficus-indica* mucilage did not negatively affect the bread doughs’ biological leavening. Formulated bread showed higher antioxidant activity than usual (control) and received a general appreciation in the sensory panel (Liguori et al., 2020).

Production of functional pasta by utilizing *Opuntia* spp. may provide an opportunity to create new healthy foods without impaired organoleptic and physical properties. Different approaches like the ELECTRE III methodology can evaluate the combination of *Opuntia* addition quantity and process conditions to produce new products (Micale et al., 2017). Fortification of durum wheat pasta with *Opuntia* cladodes offers developing foods with proven health properties. Its use exerted blood cholesterol and glucose-lowering potentiality (by reducing bioaccessibility of the sterol and starch digestibility) and prevention against atherosclerosis or inflammatory diseases. Also, fortifications with *Opuntia* fruits and cladodes were associated with preventing age-related metabolic disorders, hyperglycemia, and maintaining normal weight (Aiello et al., 2018; Attanzio et al., 2019). When pasta was supplemented with cladodes extract up to 20% (v/w), the quality (swelling index, cooking loss, dry matter) and sensory analysis proved to be satisfactory (Attanzio et al., 2019). Gluten-free pasta base was prepared with rice and field bean flour blends at a ratio of 2:1 and enriched with *Opuntia ficus indica* (L.) Mill. fruit at different amounts (2.5–15%). The quantity of free phenolic acids (mainly caffeic, syringic, 4-OH-benzoic, coumaric, ferulic), the sum of phenolics, and antioxidant capacity were positively correlated with incorporation level. Obtained pasta and other possibly derived gluten-free products may improve the quality of health and life of coeliac patients (Oniszcuk et al., 2020).

Enrichment with cactus plants was influential on the nutrition, texture, flavour, and color of the cookies and biscuits. Recently, prickly pear peel flour (substituting wheat flour by 20–30% ratio) was used as an innovative ingredient to develop fibrous structure, technological properties, flavour retention, antioxidant power, and sensorial attributes of biscuits (Bouazizi et al., 2020). When cookies were enriched with cactus cladode flour, the ash, total dietary fibre, potassium, magnesium, and calcium contents increased. The oxidative stability was also better than plain control cookies. Compatibly, higher DPPH radical scavenging and reducing power antioxidant capacities were observed following the enrichment (Msaddak et al., 2015). The protein amount may decrease or retain, and the appearance color may darken or remain the same, depending on the inclusion level. However, observed noticeable hardness in the texture, lower expansion ratio, and the greater thickness of cookies were associated with the higher water absorption capacity and lower gluten content resulting from the increased amount of fibre (Sáenz et al., 2002; De Wit et al., 2015). It has been proven that extracted alcohol-insoluble solids and skins (peels) of cactus pear could support the contents of sugar (glucose and fructose), minerals, dietary fiber, and phenolic compounds. Water and fat holding capacity were also increased when they were included in food systems (El-Shahat et al., 2019).

The instant controlled pressure drop (DIC) method could be an alternative technology to value prickly pear peels as a by-product. This treatment provided highly preferred starch-free crispy snacks with improved textural characteristics (expansion, cutting force) and remarkable amounts of phenolics,  $\beta$ -carotene, and antioxidant capacities (Namir et al., 2017).

*Opuntia* derivatives' utilization through an extrusion process, particularly with cereals, is another alternative for enriching or creating innovative products. Adding seed powder (*Opuntia dillenii*) was proven to enhance rice-based extrudates' nutritional, functional, and structural properties. Fibre, total phenolic, and flavonoid contents and DPPH and ABTS radicals scavenging activities were improved, especially using higher seed powder inclusion levels (15–20%). Breaking strength and expansion ratio significantly decreased, while water absorption and solubility capabilities and oil absorption index increased compared to control (without seed powder). In the meantime, it should also be noticed that the protein and total carbohydrate composition of the extrudates were reduced (Rayan et al., 2018). From this aspect, the nutritional-balance could be supported via some other supplementations rich in protein.

The whole yellow-orange fruit of *Opuntia ficus-indica* was added to rice or corn grits up to 10%, and the mixture was extruded. Fruit enriched snacks displayed a higher amount of flavonols, especially isorhamnetin glycosides, and better nutritional characteristics than those based on only rice or corn. Extrusion did not alter total flavonols content in the extrudates indicating their resistance against applied extrusion conditions. Since flavonols are abundant only in the fruit's peel, the inclusion of peels or whole fruit was recommended to improve bioactivity and nutritional value (Moussa-Ayoub et al., 2015). Similarly, polyphenol, betacyanin, betaxanthin contents, and maize grit extrudates' antioxidant activity increased with an additional amount of encapsulated red cactus pear powder. However, the extrusion process caused degradation in these bioactive compounds due to destructive thermal and pressure effects.

In contrast, antioxidant activity was enhanced probably because of the further release of some active substances (Ruiz-Gutiérrez et al., 2017). Extruded maize flour fortified with cactus pear exhibited higher water absorption indices and apparent densities but lower expansion ratios, as reported for rice flour previously by Sarkar et al. (2011). Authors suggested that radial expansion decrease upon cactus fruit fortification might be due to elevated sugar and viscosity levels. Also, dilution of starch by other polysaccharides (like fibre) competing with starch for water and restricting gelatinization might be another factor. Low expansion ratio and high density are undesired because of adverse effects on texture in snacks. Lately, greater expansion rate and lower apparent densities were obtained by fortification of mild-range cladode (nopal) flour (5%) without any use of xanthan gum in rice starch-based snacks (Anchondo-Trejo et al., 2020).

### 3 Conclusion

The variety of bioactive elements, nutrients, and their functional properties enable *Opuntia* spp. cladodes and pear fruits are ideal candidates for health-promoting food and supplements production. Incorporation or conversion of their nutritive, technological, and functional properties into other food systems may provide nutritionally, quality, shelf-life stable (or prolonged), attractive, and innovative products. However, new approaches conducted on industrial exploitation of these products should be supported by environmentally harmless techniques. Besides creating innovations, other strategies may target low-cost sustainable technologies of manufacturing/processing.

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# Chapter 47

## *Opuntia* spp. in Cosmetics and Pharmaceuticals



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**Abstract** The bioactive ingredients derived from various plants have commonly therapeutic potentials for different diseases. *Opuntia* spp., belonging to the *Cactaceae* family, carry a significant number of bioactive compounds with applications in food, drug, and cosmetics industries. This chapter aimed to evaluate the use of bioactive compounds derived from *Opuntia* spp. applied in cosmetics and drug industries.

**Keywords** Bioactive compounds · Nutraceuticals · Cosmeceuticals · Pharmaceuticals · Applications

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## 1 Introduction

Nutraceuticals, a portmanteau of the words ‘nutrition’ and ‘pharmaceutical,’ is defined as “the phytochemicals if they derive from a food of vegetal origin, and as the pool of the secondary metabolites if they derive from a food of animal origin, concentrated and administered in the more suitable pharmaceutical form” (Daliu et al., 2019; Durazzo et al., 2019; Santini et al. 2018; Santini & Novellino, 2018; Santini & Novellino, 2017a, b). Nutraceutical applications are intensively investigated to counteract different diseases, including cardiovascular diseases, cancer, and diabetes. Nutraceuticals are obtained from food of vegetal or animal origin. The current interest and ongoing worldwide research aim to shed light and fully clarify their mechanism of action, safety, and efficacy by substantiating their role using clinical data (Daliu et al., 2018). Nutraceuticals are a challenge for the future of prevention and therapy and a triggering tool in the medicine area.

The interest of consumers and the market in naturally-derived remedies and less synthetic pharmaceuticals is increasing, and the attention is more strongly addressed on these food-derived products. Biologically active compounds have received attention as ingredients in food products. Functional food is often used to refer to food that can provide health-enhancing effects in addition to the usual nutritive value of the food (Singh, 2019).

Cosmeceuticals are defined as compounds between cosmetic and pharmaceutical products, whose internal and external use is recommended to improve skin and hair’s beauty and health (Milam & Rieder, 2016; Draelos, 2019). It is worth mentioning the recent review of Espinosa-Leal and Garcia-Lara (2019) on new active ingredients from natural products for cosmeceutical applications. Given the significant advances in identifying phytochemicals and considering the traditional use of medicinal plants, research has led to eliminating the health and economic challenges. The extraction of bioactive compounds from agri-food products and by-products to be employed as active principles is addressed towards different industrial fields, such as food, feed, cosmetics, biomedical and agronomic applications.

## 2 *Opuntia* spp. in Cosmetics and Pharmaceuticals

In the last years, the research on bioactive compounds and potential beneficial properties of *Opuntia* spp. has led to a growing interest in the new opuntia-based-products. Nowadays, some types of products are present. Examples of market semi-finished products comprise pear pulp, prickly pear juice, flowers of prickly pear, prickly pear seed oil, prickly pear seed flour, dried fruit, and prickly pear puree. The presence of pectins and mucilage in cladode and peculiar carbohydrate fraction (glucose and galacturonic acid, minor amounts of arabinose, galactose, mannose, xylose and rhamnose, and also a trace of fucose) and flavonoids such as

rutin, quercetin and isoquercetin, isoharmetin and kaempferol (Ginestra et al., 2009) make this plant of great interest to the cosmetics industry.

In Table 47.1, studies on cosmetics and pharmaceuticals applications, having as main ingredients *Opuntia* spp. are reported. It is worth mentioning the work of Damasceno et al. (2016) showed that developed formulations containing 1 and 3% of *Opuntia ficus-indica* hydroglycolic extract enhanced the skin barrier effect by reducing transepidermal water loss up to 4 h after application. Ribeiro et al. (2015) produced and characterized cosmetic nano-emulsions containing *Opuntia ficus-indica* (L.) mill extract as a moisturizing agent. Robert et al. (2020) studied the influence of gelation on the retention of purple cactus pear extract in microencapsulated double emulsions. Double emulsions with an external aqueous phase gelled with gelatin protected betanin of a purple cactus pear extract from degradation. They can represent promising potential fat replacers in food products consumed at temperatures under the gelatin melting point.

Otálora et al. (2016) utilized an ionic gelation method to encapsulate the betalain pigment for the stabilization of *Opuntia*'s biologically active ingredients, the results of which found low storage stability and no protection at high relative humidity. The same researchers within another work extracted betalains from *Opuntia* fruits to micro-encapsulate the pigments through spray drying as a stabilization strategy using maltodextrin+cladode mucilage (MD-CM) and MD alone, followed by characterization by various methods (Otálora et al., 2015). According to the analysis of pigment storage stability, the total dietary fiber level was elevated, suggesting such microencapsulates as a potent functional additive as a natural colorant in the food industry. Fernández-López et al. (2018) found an elevation in *Opuntia* fruit-derived

**Table 47.1** Applications of *Opuntia* spp. in cosmetics and pharmaceuticals

<i>Opuntia</i> spp.	Activity	Specificities of activity	Reference
Fruit	Hepatoprotective effect	The administration of taxifolin reduced hepatic damage in rats by maintaining the GSH level	Kim et al. (2017)
Cladodes	Protective effect on the skin	The administration of diglycosideisorhamnetin-glucosyl-rhamnoside (125 ng/mL) exhibited anti-inflammatory activity in rats via pro-inflammatory prevention cytokines of IL-6 and TNF- $\alpha$ as well as the suppression of COX-2 potential	Antunes-Ricardo et al. (2015)
Cladodes	Anti-inflammatory effects	The cactus cladode extract (100 mg/mL) administration decreased erythema formation, transepidermal water loss, and epidermal thickness in mice	Park et al. (2017)
Cladodes	Antioxidant and antibacterial activities	The administration of Cladode extractable phenol (EP) and MA fractions inhibited the growth of <i>Klebsiella pneumonia</i> , <i>Listeria monocytogenes</i> , and <i>Enterococcus faecalis</i>	Aruwa et al. (2019)
Cladodes	Moisturizing effect	<i>Opuntia ficus-indica</i> (L.) Mill extract increased the skin hydration after 5 h by elevating the skin barrier impact	Damasceno et al. (2016)



betaxanthin stability throughout the use of the microencapsulation method of spray drying. Reportedly, *Opuntia* mucilage was introduced as a candidate to replace the suspending agents of sodium carboxymethylcellulose (NaCMC) in suspension formulations (Gebresamuel & Gebre-Mariam, 2013).

Prickly pear extract is also used to prepare functional food, i.e., pasta, bread, biscuits, yogurt. Kharrat et al. (2018), by studying the substitution of some synthetic additives by a natural extract from red prickly pear (*Opuntia stricta*), produced and tested salami. The authors reported that the color, taste, and texture of salami prepared with 2.5% of prickly pear extract are markedly more appreciated by panelists from the sensorial analysis. Parafati et al. (2020) evaluated the addition of prickly pear peel flour to bread dough as a source of nutrient and bioactive compounds. The formulation containing prickly pear peel flour at 10% showed the highest values in terms of the leavening dough capacity and bread specific volume and received the best sensory evaluation score. Liguori et al. (2020) showed how the addition of fresh *Opuntia ficus-indica* mucilage (in substitution to water) did not influence the biological leavening of the doughs, and on the other hand, showed the biological role of the cactus mucilage because their antioxidant activity was higher than that of control wheat bread. Attanzio et al. (2019) studied the quality, functional and sensory properties of pasta fortified with extracts from *Opuntia ficus-indica* cladodes and showed its promising healthy properties, such as blood cholesterol- and glucose-lowering capabilities.

Oniszczyk et al. (2020) produced an innovative gluten-free pasta from rice-field bean flour enriched with 2.5–15.0% of prickly pear fruits. This fortified pasta showed high content, especially of phenolic compounds, at levels of 12.5% and 15.0% of the additive (Oniszczyk et al., 2020). The current research of Dias et al. (2020) concluded how nano-encapsulated *Opuntia* extracts could also be used to supplement yogurt-like fermented soy with betalains, imparting their health benefits without changing the color.

Prickly pear seed oil is produced from prickly pear fruit waste product. The seed oil obtained from wild-grown plant fruits was characterized for oil yield, volatile compounds, fatty acids profile, *in vitro* antioxidant activity, and total phenolic content (Karabagias et al., 2020). The oil had a rich aroma, mainly due to high levels of aldehydes and alcohols, whereas it had a high *in vitro* antioxidant activity and total phenolic content. The authors suggested that the seed oil could be used as a beneficial by-product in different food systems as a flavoring, antioxidant, and nutritional agent, in addition to its current use in cosmetics.

Márquez-Lemus et al. (2019) explored the possibility of obtaining a liquor by maceration of the peeled fruit and then characterized the liquor by determining the phenolic content, volatile aromatic profile, antioxidant activity, and by sensory evaluation. Results highlighted that the liquor obtained after 2 days of maceration was characterized by the highest consumers' acceptance, the content of volatile compounds, and antioxidant activity, whereas quercitrin was the most abundant phenolic compound in the liquor. According to the authors, results from this study suggested that the *Opuntia* liquor was a rich source of phenolics and aromatic compounds and it would be a drink with the probability of being commercialized.

Concerning consumer perception, it is worth mentioning the work of de Albuquerque et al. (2019), which investigated Brazil consumer perception and use of nopal (*Opuntia ficus-indica*). Brazilians accept nopal as food concerning their functional properties, even if it does not make part of the food culture of this country, as well as the positive acceptance of Mexican nopal cookies, was reported.

### 3 Conclusion and Future Remarks

*Opuntia* spp. is rich in compounds used in the pharmaceutical and cosmetic industries. Recent research in *Opuntia* spp. has led to great opportunities in the ethnobotanical, nutritional, and medicinal areas. *Opuntia* is expected to be used in various industries, including food and pharmaceutical industries, to be an inexhaustible source of products.

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# Chapter 48

## Food and Non-Food Applications of *Opuntia* spp. Seed Oil



Maryna de Wit and Arno Hugo

**Abstract** Plant extracts are essential substances and explored for their natural active ingredients, which have different properties including nutritional, antioxidant and healing. *Opuntia* spp. are primarily grown as a fruit crop that generates huge amounts of seeds from which oils can be extracted. The seed oil is rich in polyunsaturated fatty acids, phenolics, and vitamins and included in the human diet to contribute to health. It has also been used in traditional folk medicine because of its antioxidant, anti-inflammatory and antimicrobial activities. It can also potentially be used by the food industry to manufacture natural or green safe food with an extended shelf-life. *O. ficus-indica* oil was found to be effective in cutaneous wound healing, while the antimicrobial effect prevented infections. The oil is a valued cosmetic ingredient because of its skin and hair hydration action. Linoleic acid is an essential fatty acid and a precursor of arachidonic acid biosynthesis, which is the substrate for eicosanoid synthesis. Linoleic acid has beneficial properties for the skin and also has hypocholesterolemic effects. Polyunsaturated fatty acids alleviate symptoms of coronary heart disease, stroke and rheumatoid arthritis. Sterols lower blood LDL cholesterol. This chapter reports extensively on the composition of *Opuntia* seed oils from different species and its food and non-food applications.

**Keywords** Antioxidant · Antimicrobial · Cosmetic · Fatty acids · Nutraceutical · Pharmaceutical · Medicinal · Trans-epidermal water loss

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## Abbreviations

ABTS	3-Ethylbenzothiazoline-6-sulphonic
ALA	$\alpha$ -Linoleic acid
CAT	Catalase
DPPH	2,2-diphenyl-1-picrylhydrazyl
EFA	Essential fatty acid
FA	Fatty acids
FAD	Fatty acid desaturase
FFA	Free fatty acids
FRAP	Ferric reducing
GIT	Gastro-intestinal tract
IV	Iodine value
LDL	Low density lipoprotein
LLL	Trilinolein
MUFA	Monounsaturated fatty acids
NMF	Natural moisturizing factor
OLL	Dilinoeoyl-oleoyl-glycerol
ORAC	Oxygen Radical Absorbance Capacity
OSI	Oxidative stability index
PUFA	Polyunsaturated fatty acids
PV	Peroxide value
RI	Refractive index
ROS	Reactive oxygen species
SACPD	Stearoyl-acyl carrier protein denaturase
SFA	Saturated fatty acids
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substance
TEWL	Trans-epidermal water loss
TPTZ	2,4,6-Tri(2-pyridyl)-s-triazine
UFA	Unsaturated fatty acids
UV	Ultraviolet
VLC SFA	Very long chain saturated fatty acids

## 1 Introduction

Vegetable oils are essential in the human diet, since they provide energy. Vegetable oils are nutrient-rich as they contain essential fatty acids, phytosterols, pigments, phenolic compounds and fat-soluble vitamins (Brahmi et al., 2020). These oils are used in the food industry to produce margarines, chocolate, cooking oil and in the cosmetic industry to produce soaps and lotions. Oils also have beneficial effects on health. Many properties of different oils have been reported including

cardiovascular disease preventative, hepatoprotective, cytoprotective against oxidized cholesterol derivatives (oxy-sterols), the levels of which, increase with the different age-related diseases namely cardiovascular, neuro-degenerative and ocular diseases. Some oils prevent prostate adenomas. Recently, some oils showed the ability to chelate heavy metals from polluted environments (Brahmi et al., 2020). Plant seeds are excellent sources of phytochemicals for not only the nutritional and pharmaceutical industries, but also industrial applications. New oil sources of under-exploited seeds are important sources of phytochemicals (Tlili et al., 2011).

## 2 Cactus Pear (*Opuntia* spp.) Seed Oils

The *Opuntia* cactus has recently been recognized as a functional food and a source of nutraceuticals or parts of food that provide medical and health benefits and will either prevent or treat a disease (Ondarza, 2016).

### 2.1 History

Most of the plant parts benefit humans and animals both internally and externally and have been used throughout the world. For instance, in traditional medicine, *Opuntia ficus-indica* has been used for the treatment of burns. The Aztecs extracted the milky juice from the plant and mixed it with honey and egg yolk to provide an ointment to treat burns. Prickly pear cactus has been used for wounds, oedema, hyperlipidemia, obesity and catarrhal gastritis. In Mexican traditional medicine, prickly pear cactus (nopal) is used for the treatment of diabetes and high cholesterol. Alcoholic extracts have been indicated for anti-inflammatory, hypoglycemic, and antiviral purposes. The Chinese dressed abscesses with the fleshy pad of the plant. The American Indians used the fruit for food and made syrup from it to treat whooping cough and asthma (Shutes, 2020).

Only recently, research has begun to show just how much the seeds of the prickly pear can benefit the skin. Within the edible part of each prickly pear fruit, there are numerous seeds. The seed amount can vary from 30–40% on a dry weight basis. These seeds contain nutrient-rich oil that is extracted and then used on the skin. Today, oil can be pressed from the seeds and then used as a carrier oil or ingredient in cosmetics and skincare applications (Shutes, 2020).

Many plant essential oils have beneficial biological properties such as antioxidant and antimicrobial activities. These bioactive compounds (oils and its constituents) increase the value of *O. ficus-indica* as a crop (Zito et al., 2013). From the seeds, alcohols, aldehydes and ketones, fatty acids, hydrocarbons, phenolic compounds and terpenes can be extracted (Zito et al., 2013). In the production of *O. ficus-indica* juice, masses of seeds are annually discarded. This could lead to the use of cactus seeds as a new source of oil and meal containing beneficial compounds

(Zito et al., 2013). *O. ficus-indica*, *O. stricta*, and *O. dillenii* are commercially grown for both food and feed.

Cactus pear (*O. dillenii*) has gained increased consumer interest as being categorized as a functional food (Ali-Alsaad et al., 2019). This is due to its nutritional value with regard to its fatty acid content as well as its health benefits. *O. dillenii* seed oils also contain phenolic acids, flavonoids and tannins, which all considered as antioxidants in the pharmaceutical industry. The fatty acid composition adds to the nutritional value since some are essential fatty acids. Supplementation of food products with cactus pear seed oil represents value-added products, with increased antioxidant activity. The level of fatty acids in *O. dillenii* seed oil is higher than that in sunflower, grape, and sesame seed oils. *Omega-6* linoleic acid and arachidonic acid presented inhibitory effects on metastatic cancer cells (Ali-Alsaad et al., 2019).

### 2.1.1 Components of *Opuntia* Seed Oil

The chemical components found in prickly pear seed oil, that support the health and texture of the skin, was examined in a studies by Ramadan and Mörssel (2002, 2003a, b), where total lipids were found to be 98.8 g/kg dry weight. It was found that the fatty acid profile of seed oil evinces the lipids as a good source of the essential fatty acids (linoleic and oleic), wherein the ratio of linoleic to oleic was about 3:1. Linoleic acid was the most abundant fatty acid, followed by palmitic and oleic acids. These authors suggested that the levels of total lipids may depend on fruit cultivar, degree of ripeness, fruit processing and its storage conditions. As for fat-soluble vitamins, Ramadan and Mörssel (2002, 2003a, b) found vitamin E level of 0.04% of total lipids in the seed oil.  $\gamma$ -Tocopherol was found to be the main form of vitamin E, followed by  $\alpha$ -tocopherol. Vitamin A, in the form of  $\beta$ -carotene, accounted for less than 0.42 g/kg in seed oil. Vitamin K1 was also present at a level of 0.05% of the total lipids. High levels of sterols were found, which made up 9.33 g/kg of seed oil.  $\beta$ -Sitosterol was the sterol marker, which comprised 72% of total sterol content in the seed oil. It was followed by campesterol. Stigmasterol, lanosterol,  $\Delta^5$ -avenasterol, and  $\Delta^7$ -avenasterol (Ramadan and Mörssel (2002, 2003a, b).

Ramadan and Mörssel (2002) also found prickly pear seed oil to be exceptionally rich in fatty acids, especially in linoleic acid. With regard to its lipid profile, *Opuntia* seed oil was considered exceptionally rich and comparable to grape seed oil. In a study that tested four different coloured fully ripened fruits (red, orange, yellow, and green) and its seed oils, it was found that the level of linoleic acid in one colour of fruit did not necessarily mean similar levels of other fatty acids. For example, while linoleic levels ranged from high to low as follows: 63.1% for orange, 62.1% for yellow, 61.8% for green, and 58.7% for red, oleic acid levels ranged from high to low as follows: 24.3% for red, 20.9% for yellow, 16.3% for green, and 15.2% for orange (Morales et al., 2012; Chougui et al., 2013). Prickly pear seed contained 403 mg/kg of vitamin E, mostly in the form of  $\gamma$ -tocopherol. The seed oil is made stable by the



tocopherol presence. High levels of vitamin E detected in these oils may contribute to its greater stability against oxidation (Ramadan and Mörsel 2002, 2003a, b).

Polyphenols are abundantly found in the cactus pear. The growing interest in polyphenols resulted from its antioxidant potential. These polyphenols exhibit health benefits such as the prevention of inflammation, cardiovascular dysregulation, and neurodegenerative diseases. Polyphenols are free radical scavengers and have been proven to possess anti-carcinogenic activity. All parts of the cactus pear plant are rich in phenolics from different families, such as various flavonoids and phenolic acids. Cactus pear seeds contain high amounts of both, ranging from 48 (red fruit) to 89 (orange fruit) mg/100 g, and include feruloyl derivatives, tannins, and sinapoyl diglucoside. In the study that examined the four different coloured cactus fruits, the phenolic profile of the seeds displayed a high complexity, with more than 20 compounds detected. Among these, three isomers of feruloyl-sucrose and sinapoyl-diglycoside were identified. Significant correlations were found between phenolic content in the defatted seed extracts and their antioxidant activity. Significantly higher values for all detected phenolic compounds were found in the seed extract of the orange fruit. The samples presenting the highest antioxidant activities also had the highest phenolic, tannin and flavonoid contents (Morales et al., 2012; Chougui et al., 2013).

Although the oil yield is relatively low (de Wit et al., 2016), its content of functional ingredients makes it a valuable oil which is currently one of the most expensive oils in the world. At about \$2000 per litre, prickly pear seed oil is the most expensive carrier oil on the market (Shutes, 2020). *Opuntia* seed oil is rich in beneficial health compounds, e.g. unsaturated fatty acids (UFA), phytosterols, fat-soluble vitamins (vitamin E),  $\beta$ -carotene and antioxidative phenolic compounds (Koubaa et al., 2016). Tocopherols, for instance, are highly bioactive natural antioxidants (Gharby et al., 2020). Depending on the origin and the genetics (specie) of the fruit, as well as the extraction method, oil content may vary from as low as ~2% to as high as ~17%. Cactus pear seed oil have a high level of unsaturation (82%), made up of mainly linoleic (65.3%) and oleic (15.0%) acids (de Wit et al., 2016, 2017, 2018). Saturated fatty acids (17.7%) are found in lower concentrations and consist mainly of palmitic (13.6%) and stearic (3.19%) acid (de Wit et al., 2016, 2017, 2018).

Karabagias et al. (2020) showed that cactus pear seed oil is a matrix of a rich aroma, containing aldehydes (62.7%), alcohols (9.13%), hydrocarbons (5.06%), ketones (4.38%), esters (2.85%), and acids (2.70%), as well as phenolic compounds. The highest contents of oleic acid and butyric acid was recorded in Greek *O. ficus-indica* seed oil. Seeds and oil from Turkish *O. ficus-indica* contained high contents of linoleic acid, fibre and minerals, such as Ca, K, Mg, P and Na, making them nutraceutic agents (Özcan & Al Juhaimi, 2011). Farah et al. (2018) reported on the nutritional value of polyunsaturated fatty acids (PUFA) extracted from Algerian *O. ficus-indica*.

Cultivar type, temperature and harvest time influence pH, °Brix, vitamin C, sugars and lipid content of the cactus pear fruit (de Wit et al., 2010). Variations in fatty acids profiles e.g. that of linoleic and oleic acids were also influenced by cultivar and location interactions which provide unique properties to the oils (de Wit et al.,

2016; Ramírez-Moreno et al., 2017).  $\gamma$ -Tocopherol found in the seeds of *O. microdasys* (Lhem.) and *O. macrorrhiza* (Engelm.) from Tunisia are in line with that found in *O. joconostle* and *O. matudae*. The increased tocopherol contents could have been triggered because of a biochemical response to the high PUFA levels, as tocopherols are effective lipophilic antioxidants (Chahdoura et al., 2015).

Vegetable seed oils are the most important dietary sources of tocopherols. Plant essential oils have beneficial properties such as containing antioxidants. It is the presence of these essential compounds that increases the value of *O. ficus-indica* (cactus pears) as a crop (Zito et al., 2013). The *O. ficus-indica* cactus was described as a gold mine and a source of immense wealth (Farah et al., 2018). The seed oil is valuable because of its high contents of the essential *omega*-6 fatty acid, linoleic acid, vitamin E and sterols (Ennouri et al., 2005). The presence of valuable bioactive substances such as PUFA, tocopherols, sterols, phenolics and carotenoids in edible cold-pressed oils define oil quality (Fernández-Martínez et al., 2004). In a study by de Wit et al. (2020), the fatty acid profile and oleo-chemical quality properties of cold pressed cactus pear seed oil, as well as its oxidative stability at two storage temperatures, 25 °C and 30 °C, over a 12-months period was investigated. The study indicated that there was less primary oxidation in the sample that was stored at a higher temperature (30 °C) than the sample stored at 25 °C. This was in contradiction to results in literature where it was stated that oxidation will occur at a slower rate at a lower temperature. These values indicated that the sample stored at 25 °C would be more susceptible to oxidation than the sample at 30 °C. It was postulated that this discrepancy might be attributed to the possible better release of antioxidants at the higher storage temperature. This trend was also observed in recent studies by du Toit et al. (2018a, b), where high antioxidant content and activity, as well as its retention in processed products from cactus pear fruit pulp, seeds and cladodes, especially during heat processing were observed. It might also be an adaptation of this specific species to survive at higher temperatures and will therefore have a more stable profile at 30 °C than at 25 °C. The predicted shelf-life was found to be shorter than the actual shelf-life, as determined by the comprehensive 1-year shelf-life evaluation. Both oils were very stable and it was concluded that storage time had a more pronounced effect on oil quality than storage temperature (de Wit et al., 2020).

In a recent study by de Wit et al. (2021), the main findings pointed out that cold pressed cactus pear seed oils yielded lower oil contents than chemically extracted oils, while the green-coloured fruit had the highest yields which indicate the effect of cultivar and species. Quality parameters of the oils were strongly influenced by the fatty acid composition and physicochemical properties. Large differences in oil content, fatty acid composition as well as physicochemical properties [iodine value (IV), peroxide value (PV), refractive index (RI), tocopherols, ORAC (antioxidant activity), % free fatty acids (%FFA), Oxidative Stability Index (OSI) and induction time] were observed. Oil content, PV, %FFA, RI, IV, tocopherols, ORAC and *p*-anisidine (*p*-AV) value were negatively correlated with OSI. In general, lower PUFA and saturated fatty acid (SFA) contents and higher mono-unsaturated

(MUFA) contents were observed in the cold pressed oils compared to the chemically extracted oils, giving it a more desirable profile from a health perspective. The cold pressed oil was found to possess higher RI, IV and  $\rho$ -AV than their chemically extracted counterparts, while the PV, FFA and OSI of cold pressed oils were lower (shorter). Cold pressed oils are more likely to undergo secondary oxidation in contrast to the chemically extracted oils, which will be more prone to primary oxidation. Tocopherol content and antioxidant activity contributed to stability and quality. Extrapolated induction times indicated a shelf life of up to ~3.5 years when stored at 25 °C.

### 2.1.2 Comparison to Other Oils

Cactus pear seed oil is similar in composition to other vegetable oils such as grape seed and cotton seed oils (Labuschagné & Hugo, 2010). It is especially important because of its essential *omega*-6 fatty acid (linoleic acid), vitamin E (1000 mg/kg) and sterol contents (10 g/kg).  $\gamma$ -Tocopherol is higher in cactus pear seed oil than that in corn and soy bean oils. It is important to note that corn and soy bean oils have been classified as some of the best sources of  $\gamma$ -tocopherol, therefore cactus pear seed oil should be highlighted as a valuable source as well (Loizzo et al., 2019).

## 3 Quality, Stability and Oxidation

Seed oil quality depends mainly on the fatty acids, triacylglyceride composition and antioxidant properties (Hssaini et al., 2020). The balance between *omega*-6 and *omega*-3 fatty acids, two classes of essential fatty acids, is important for oil quality, especially in human nutrition. A lower *omega*-6: *omega*-3 ratio (< 5) is important to reduce cancer, increase bone health and reduce cardiovascular diseases. A lack of *omega*-3 fatty acids had been linked to some health problems, e.g. blood lipids, auto-immune, cardiovascular and inflammatory diseases.

Saturated fatty acids (SFA) are stable against oxidation. Unsaturated fatty acids (UFA) are prone to instability at high temperatures and other oxidative reactions that cause the development undesirable hydroperoxides, aldehydes and ketones. The double bonds of the fatty acids react with oxygen and produce free radicals, which are implicated in processes of ageing, injuries and certain diseases. Fatty acid oxidation causes off-flavours, while its autoxidation reduces its shelf-life (Labuschagné & Hugo, 2010; de Wit et al., 2017, 2020, 2021). Phenolics are linked to oleic acid stability in preventing oxidation and prolonging oil shelf-life (Barba et al., 2017). Lipid oxidation has an impact on quality and nutritional value of food. It causes rancidity, off-flavours and off-tastes as well as colour changes. Cactus pear seed oils were effective in suppressing oxidation of cakes. This was due to the “added” antioxidants that prevented lipid peroxide formation (Ali-Alsaad et al., 2019).

## 4 Functions and Applications of Individual Oil Compounds in the Food, Cosmetic, Nutraceutical and Pharmaceutical Industries

Cactus pear seed oil is rich in fatty acids, namely SFA, MUFA and PUFA, some of which are essential fatty acids, while phytosterols consist mainly of  $\beta$ -sitosterol and tocopherols ( $\gamma$ -tocopherol). Linoleic acid,  $\alpha$ -linolenic and  $\gamma$ -linolenic acid are all essential fatty acids and should be included in the human diet (El-Said et al., 2011). These fatty acids cannot be produced naturally within the body and must be acquired through diet. Humans lack the desaturase enzymes required to produce these fatty acids (Ramadan & Mörseel, 2003a, b). Unsaturated fatty acids (UFA) e.g. the mono-unsaturated oleic acid (C18:1) as well as the polyunsaturated linoleic acid (C18:2) and  $\alpha$ -linolenic acid (ALA, C18:3) have the property to lower LDL cholesterol (serum low-density lipoprotein) and thus may lower total cholesterol, reducing the risk of cardiovascular diseases (Labuschagné & Hugo, 2010).

Oleic acid has a hypocholesterolemic effect which makes it a preferred edible fatty acid. In the fatty acid biosynthesis pathway, oleic acid (which is synthesized from stearic acid by the Stearoyl-Acyl Carrier Protein Denaturase, SACPD) is also considered as the precursor of linoleic acid (by the Fatty Acid Desaturase, FAD<sub>2</sub>). In *O. dillenii*, oleic acid content is low, because of the high FAD<sub>2</sub> activity (converting most oleic acid into linoleic acid). Both SACPD and FAD<sub>2</sub> enzymes have a major effect on the levels of oleic acid and stearic acid in seed oil. The accumulation of linoleic acid in *O. dillenii* is, thus, explained by the increase/decrease of SACPD/FAD<sub>2</sub> expression (Ali-Alsaad et al., 2019) (the high FAD activity led to most of the oleic acid being converted into linoleic acid).

Saturated fatty acids, such as myristic, palmitic and stearic acids were initially considered to raise LDL cholesterol levels. All SFA are, however, not equal in their cholesterol raising potential. Stearic acid was found to have a neutral effect on the blood serum LDL cholesterol concentration and, therefore, has no cholesterol effect on health as indicated by Ali-Alsaad et al. (2019). Lauric, myristic and palmitic acids increased plasma cholesterol concentration (Ulbricht & Southgate, 1991). The fruits of *O. ficus-indica* and *O. dillenii* have hypocholesterolemic effects due to the fatty acids in the seed oils as well as anti-hyperglycemia, anti-inflammatory, and analgesic effects (Ghazi et al., 2013).

Seed oils from *O. ficus-indica* and *O. dillenii* cultivars from Morocco contained high levels of linoleic acid, with an exceptionally high level observed in *O. dillenii* oil. Linoleic acid, an essential fatty acid, is a precursor of arachidonic acid biosynthesis, which, in turn, is the substrate for eicosanoid synthesis. Linoleic acid also has hypocholesterolemic effects. Linoleic acid is present in phospholipids, cell membranes and abundant in the brain and liver (Ali-Alsaad et al., 2019). It has also been reported to have beneficial properties for the skin and, therefore, used in the cosmetic industry. Linolenic acid (*omega*-3) is important in the secondary prevention of cancer (Loizzo et al., 2019). Furthermore, PUFA can alleviate symptoms of diseases such as coronary heart diseases, strokes and rheumatoid arthritis. Sterols

have also been added to vegetable oils to act as functional food and proven to lower LDL cholesterol.  $\beta$ -Sitosterol is the principal steroid found in cactus pear seed oil. Vitamin E is represented as  $\gamma$ -tocopherol, which might contribute to these oils' oxidative stability. This effect was also noted in a recent study performed by de Wit et al. (2020).

Cactus pear fruit and its products are utilized in food and nutritional supplements. Oils possess UFA (Matthäus & Öscan, 2011; Chougui et al., 2013), antioxidant, and antimicrobial activity (Zito et al., 2013) as well as cardioprotective, anti-thrombotic, anti-inflammatory, anti-arrhythmic, hypolipidemic and anti-hyperglycemic properties, all important for the pharmaceutical and food sectors (Karabagias et al., 2020). Antioxidant capacity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) chelating activity method predicted oxidative stability of edible oils. Ali-Alsaad et al. (2019) investigated seed oils from *O. dillenii* from Iraq and it contained stearic acid in higher concentrations than found in soy beans. It had no cholesterolic effect on health.

## 5 Functional Properties and Applications of *Opuntia* Seed Oil

### 5.1 Antioxidant

According to Stanner et al. (2004), the antioxidant hypothesis stresses that “as antioxidants can prevent oxidative damages, increased intakes from the diet will also reduce the risk of chronic diseases”. Seed oils have been added to food as natural agents to prevent microbial and oxidative activity. *Opuntia ficus-indica* and *Opuntia dillenii* exhibited high antioxidant activity, ascribed to the presence of bioactive compounds such as phenolics and PUFA. de Los Angeles Ortega-Ortega et al. (2017) reported on the antioxidant activity and anti-microbial activity of cactus pear seed oil. The phenolics contained in seed oils might also contribute to its beneficial health properties. Antioxidant activity was measured by two parameters namely antioxidant activity and scavenging capacity by ABTS (3-ethylbenzothiazoline-6-sulphonic) and DPPH methods. Extraction method had an effect on the antioxidant content and activity (de Los Angeles Ortega-Ortega et al., 2017).

Ramírez-Moreno et al. (2017) observed both antioxidant and antimicrobial activities in seed oils from *O. albicarpa* and *Opuntia ficus-indica*. Cactus pear fruit seed oils have a high degree of unsaturation, antioxidant radical scavenging properties and broad spectrum antimicrobial activity that can be applied in the food industry as “natural” or “green” safe food and to extend shelf life. Antioxidant and antimicrobial activities are important for the pharmaceutical and food industry. The DPPH test was used to predict oxidative stability of edible oils and antioxidant activity. *O. albicarpa* exhibited higher antioxidant activity than *Opuntia ficus-indica*. Interestingly, synergism between fatty acids may affect antioxidant activities.

Extraction method, solvent type, solvent polarity, bioactive compounds, selectivity of radicals as well as variety, have an effect on the antioxidant activity (Ramírez-Moreno et al., 2017).

The most recommended way to prevent and inhibit microbial growth in foods is to incorporate food preservatives. Essential oils are secondary metabolites and employed in food flavouring and food preservation (Zito et al., 2013; Ali-Alsaad et al., 2019). Antioxidative activity, measured by the DPPH method, reducing power,  $\beta$ -carotene bleaching inhibition and thiobarbituric acid reactive substances' (TBARS) inhibition, were higher in *O. macrorhiza* than *O. microdasys*. These high levels were attributed to the higher level of phenolic compounds (Chahdoura et al., 2015).

Brahmi et al. (2020) found a negative correlation between the phenolic compounds (total polyphenols and flavonoids) and the DPPH scavenging activity of *Opuntia ficus-indica* seed oil from Algeria. The authors suggested that other compounds, instead of phenolic compounds, may be responsible for the antioxidant activity of *Opuntia ficus-indica* oils or that the antioxidant activity might also depend on the structure of the phenolic molecules as well as the stage of maturity. Seed oil also exhibited strong antioxidant ability as a result of its ability to reduce oxidation. In the application of *O. dillenii* seed oil as antioxidant in cake, it was found that the seed oil had antioxidative effects on the cake, adding to the oxidative stability of the cake. At the same time, value was added to the cake by the additional contribution to antioxidant content. The seed oil can, thus, be used in food industry as natural and green safe food to extend the shelf life (Ali-Alsaad et al., 2019).

Antioxidant activity of the seed oil, as determined by the DPPH test, was found to be high. This was ascribed to the presence of phenolic compounds such as tocopherols. *O. stricta* oil also showed antioxidant activity due to phenolics (Ali-Alsaad et al., 2019). Different tests were used to screen the antioxidant activity, including FRAP (ferric reducing power), ABTS as well as the  $\beta$ -carotene bleaching test. Different tests measured different types of antioxidant functions. The  $\beta$ -carotene bleaching test involves the protection of the extract from lipid peroxidation. The FRAP value is the ratio between the slope of the linear graph for the reduction of the  $\text{Fe}^{3+}$ -TPTZ reagent compared to the plot for  $\text{FeSO}_4$ . Both red and yellow *Opuntia ficus-indica* seed oils showed promising antioxidant activity. Lower antioxidant potentials were found for *O. microdasys* and *O. macrorhiza* seed extracts (Chadoura et al., 2015).

## 5.2 Antimicrobial

Antimicrobial activity of seed oil was tested against *Eserichia coli* and *Staphylococcus aureus*. It was proven to be more effective against gram positive bacteria than gram-negative bacteria (de Los Angeles Ortega-Ortega et al., 2017). *Saccharomyces cerevisiae* (yeast) and *Candida albicans* (mould) growth were inhibited by cactus pear seed oil. It was consequently concluded that certain

compounds in cactus pear seed oil exhibit antimicrobial activity. The same antimicrobial activity was also reported for *O. stricta* and might be attributed to variable chemical compounds in oils. It was speculated that oil compounds act on different bacterial structures, while it was also suggested that whole oils have greater antibacterial activity than the mixed major components, therefore, implicating that minor components are critical for the activity and exert synergistic effects (Ramírez-Moreno et al., 2017).

In a study carried out by Khémiri et al. (2019), the antimicrobial effect was more profound against fungi than bacteria, suggesting a link between the chemical contents of the oil and the antimicrobial activity. No inhibition of *Salmonella typhi*, a gram negative bacteria, was observed. The reason for this finding lays in the outer membrane of gram negative bacteria, which restrict penetration of compounds and extrude toxins. The antimicrobial effect of the oil extracts had similar effects to other antimicrobials such as ampicillin, streptomycin and sulfamethoxazole/trime-thoprim. Cactus pear seed oils can, therefore, be developed as additives in the food, cosmetic and pharmaceutical sectors as antimicrobials and natural antioxidants.

Brahmi et al. (2020) found no antimicrobial activity against the strains tested, but ascribed this finding to the assay method used, as well as the low concentration of phenolic compounds, since a positive correlation between phenolic content and antimicrobial activity was reported. Phenolics such as tannins and flavonoids are important antimicrobial agents. The numbers and positions of the hydroxyl groups on the aromatic rings of these phenolic compounds have an effect on its toxicity to microorganisms. The antimicrobial activity not only depends on the presence of phenolic compounds, but also on the presence of secondary metabolites. Furthermore, lipophilic flavonoids can destroy microorganisms' membranes by increasing the lipid fluidity. The authors emphasized the importance of *Opuntia ficus-indica* seed oil in the nutritional and medical industries on account of their nutrients such as fatty acids and sterols (Brahmi et al., 2020). *O. stricta* seed oil possesses important antimicrobial activities (Koubaa et al., 2016). Extraction method influenced properties, e.g. phenolic content. This phenolics-rich oil exhibited high antioxidant (DPPH) and microbial properties (disc diffusion method).

### **5.3 Applications of Seed Oil in Medicinal, Pharmaceutical and Nutraceutical Industries**

#### **5.3.1 Cholesterol**

In soy beans, stearic acid, because of its neutral effect on cholesterol, is a sought-after property. Cactus pear seed oil contains higher levels of stearic acid than soy beans. Cactus pear seed oil, therefore, presents additional value that can benefit human health (Ali-Alsaad et al., 2019). Oleic acid has plasma cholesterol-lowering activity, while linolenic and linoleic acids can convert to hormone-like eicosanoids, which may be involved in blood clotting and immune response physiological

reactions (Hssaini et al., 2020). Linoleic and arachidonic acids present hypocholesterolemic effects and showed inhibitory effects against colon cancer metastatic cells. Ennouri et al. (2005) described the anti-hyperlipidemic effects and cholesterol-reducing effects from *Opuntia ficus-indica* seeds and seed oils.

### 5.3.2 Coronary Heart Disease

It was previously reported that PUFA are able to alleviate symptoms of diseases such as coronary heart disease, strokes and rheumatoid arthritis. Linoleic acid (*omega*-6, the main fatty acid in cactus pear seed oil) can be converted into eicosanoid precursors, such as leukotrienes and prostaglandins, which play important roles at blood vessel level for blood coagulation. It also has beneficial properties for skin and important in the cosmetic industry. Linolenic acid (*omega*-3) plays a role in the secondary prevention of coronary heart diseases and in prevention of cancer. Oleic acid (MUFA) has a fundamental role in many diseases, such as cardiovascular disease prevention. Cactus pear seed oil also contains very long-chain saturated fatty acids (VLCSFA) (>20 carbons). These are constituents and intermediates making up cuticular surface layers of plant tissue (Tlili et al., 2011).

### 5.3.3 Inflammation

Inflammation is the cascade of defence reactions produced by a host against external stimuli (Koshak et al., 2020). These reactions cause pain, swelling, heat and redness in the affected (inflamed) part. Inflammation causes increased blood flow and vascular permeation that results in the accumulation of fluid and inflammatory mediators such as cytokines, eicosanoids and reactive oxygen species (ROS) in the affected tissue. During the immune response, feedback mechanisms are activated through the secretion of anti-inflammatory cytokines that will inhibit pro-inflammatory signalling cascades in order to maintain homeostasis and healthy tissue (Koshak et al., 2020).

Nowadays, natural drugs are widely sought after because of its effectiveness, less side effects, and economic costs. Classic anti-inflammatory non-steroidal, selective and non-selective medicines cause severe side-effects, such as renal failure and harmful effects on the gastro-intestinal tract (GIT), and cardiovascular system. Fatty acids are some of the natural occurring plant-derived active compounds that demonstrate anti-inflammatory activity. Poly-unsaturated fatty acids are classified as *omega*-3, *omega*-6 and *omega*-9, based on the number and position of double bonds. *Omega*-6 linoleic acid is converted into arachidonic acid in mammals and also the source of inflammatory mediators' prostaglandins and leukotrienes. Consumption of oleic acid (*omega*-9) and  $\alpha$ -linolenic acid (*omega*-3) act as arachidonic acid antagonists and decrease the production of inflammatory mediators. Seed oil obtained from various *Opuntia* spp. is rich in PUFA and show antioxidant, cytotoxic, antimicrobial, antifungal, and analgesic effects. In the study of the oil from *Opuntia ficus-indica* from Saudi Arabia, the anti-inflammatory activity of the oil



was attributed to the UFA and sterols ( $\beta$ -sitosterol and campesterol). Sitosterol is known for its anti-inflammatory effect by inhibition of myeloperoxidase, IL-1B, TNF- $\alpha$  levels. UFA (*omega*-9 oleic acid) act as arachidonic acid antagonist and decrease production of inflammatory mediators such as prostaglandins and leukotrienes by acting in synergism with sitosterol to reduce inflammation (Koshak et al., 2020).

The evaluation of anti-inflammatory effects can be done with an induced oedema test, as was explained by Ammar et al. (2018). This inflammation model provokes a local inflammatory reaction and involves the release of inflammatory mediators like histamine and serotonin in the initial phase. This is followed by synthesis and release of prostaglandins in the second phase of inflammation. The reduction in the size of the oedema gives a good indication of the anti-inflammatory agents' protective action. Oxidative stress, caused by ROS, is another cause of inflammation, when it exceeds the capacity of the endogenous antioxidant system. Superoxide dismutase (SOD), catalase (CAT), and hydrogen peroxide ( $H_2O_2$ ) decomposition and glutathione provide the first line of cellular defence against toxic free radicals. Inflammation usually decrease these compounds' activities, while treatment with phenolics, mainly flavonoids, would restore the normal activities of these compounds. The anti-inflammatory effect is attributed to synergistic effects of phenolic compounds, e.g. phenolic acids and flavonoids (Koshak et al., 2020). According to Karabagias et al. (2020),  $\beta$ -sitosterol is also responsible for anti-inflammatory and angiogenic effects.

### 5.3.4 Hypoglycaemic

The hypoglycaemic effect of cactus pear seed oil was reported by Berraouan et al. (2014). The oral administration of seed oil decreased postprandial hyperglycaemia, because of the partial reduction in D-glucose intestinal absorption. The oil also prevents alloxan-induced diabetes by quenching the free radicals produced by alloxan. This was ascribed to the chemical composition of the oil, specifically linoleic and oleic acid. *Omega*-6 linoleic acid rich oil prevents alloxan-induced diabetes and increase the levels of endogenous antioxidants such as vitamin E, SOD, and glutathione. *Opuntia ficus-indica* seed oil is a promising source of health compounds, including PUFA, carotenoids and  $\gamma$ -tocopherols (Loizzo et al., 2019). Ennouri et al. (2005) described hypoglycaemic and antidiabetic effects from *Opuntia ficus-indica* seeds and seed oils.

### 5.3.5 Anti-Cancer

As already mentioned, Ali-Alsaad et al. (2019) reported that *omega*-6 linoleic acid and arachidonic acid presented inhibitory effects on metastatic cancer cells. Linolenic acid (*omega*-3) is important in the secondary prevention of cancer (Loizzo et al., 2019).

### 5.3.6 Wound Healing

Khémiri et al. (2019) researched the wound-healing and antimicrobial potential of *O. ficus-indica* L. *inermis* seed oil from Tunisia. Authors reported the use of *Opuntia ficus-indica* in traditional medicine, pharmacopeia, with the focus on the wound healing effect of *Opuntia ficus-indica* oil on full thickness skin wounds and the effectiveness in improvement of healing of laser-induced skin burns. Healing is the ability of skin to regenerate after damage was caused by injuries, burns, surgeries or ulcers. The process is triggered immediately to restore the continuity and integrity of the cutaneous barrier promptly. Acute healing is a complex process involving four steps and phases, overlapping and in succession, namely a vascular phase (haemostasis to stop loss of blood from injured vessels), an inflammatory phase, a proliferation phase (including granulation tissue formation, angiogenesis, and re-epithelialization) and scar formation (remodulation of maturing skin after wound closure). Although wound healing is a natural process, its management is important to prevent infections, scar formation, deep sores, bedsores and ulcers. Microbiota may seriously impair healing. Pathogens exhibiting multi resistance, such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Enterococcus*, *Enterobacteriaceae* and *Candida albicans* are on the increase. New therapeutic agents and phytotherapy, as alternative medicine, are being evaluated and plants with secondary metabolites and active compounds such as phenolics, flavanoids and alkaloids could serve this purpose. *Opuntia ficus-indica* cold pressed oil was able to inhibit *Enterobacter cloacae*, as well as fungal pathogens (*Candida parapsilosis* and *C. sake*). *Opuntia ficus-indica* oil had good wound healing properties and accelerated the healing process. This was attributed to the oils' richness in UFA, triacylglycerols, phytosterols and tocopherols.  $\beta$ -Sitosterols have anti-inflammatory effects, angiogenic potency, promoting local neovascularisation in the granulation tissue. Plant sterols also play an important role in stabilizing the phospholipid bilayers of cell membranes (improving cell integrity). Antioxidants, e.g. phytosterols and tocopherols inhibit free radicals, while UFA and triacylglycerols, mainly trilinolein (LLL) and dilinoeoyl-oleoyl-glycerol (OLL), protect against peroxidation and cause reconstruction of the phospholipids of the cell walls (Khémiri et al., 2019).

Linoleic acid is precursor of arachidonic acid, which is major PUFA in skin. Arachidonic acid is precursor of biologically active inflammatory mediators, namely prostaglandins, specifically thromboxone and leukotrienes that might stimulate angiogenesis, fibroplasia, and extracellular matrix remodulation. Hydration is required during wound healing. Fatty acids and triacylglycerols enhance skin hydration by reducing trans-epidermal water loss (TEWL). *Opuntia ficus-indica* oil exhibited significant antimicrobial effects against bacteria (*Enterobacter cloacae*), yeasts (*Candida parapsilosis*, and *C. sake*) and fungi (*Aspergillus niger*, *Penicillium digitatum*, and *Fusarium oxysporum*). *Opuntia ficus-indica* demonstrated both bacteriostatic and bacteriocidal actions. Free fatty acids have both bacteriocidal and antifungal properties, especially linoleic and oleic acid. The FFAs may act by inhibiting membrane enzyme activities such as glucosyl transferase or by activating autolytic enzymes in the pathogen cell wall. Phytosterols, i.e.  $\beta$ -sitosterol, could inhibit

growth of microorganisms by inducing necrosis of pathogenic cells (Khémiri et al., 2019).

#### **5.4 Applications and Functions in Food Industry**

Lipids are used as food ingredients to impart a better texture, mouthfeel and flavour. They also attribute to nutritional functions of food, i.e. provide essential fatty acids, and sources of bioactive compounds (Hssaini et al., 2020). In the application of *O. dillenii* seed oil as antioxidant in cake, it was found that the seed oil had antioxidative effects on the cake, adding to the oxidative stability of the cake. Value was added at the same time to the cake, by the additional contribution to antioxidant content. The seed oil could, thus, be used by the food industry as green and natural safe food to extend the shelf life (Ali-Alsaad et al., 2019).

#### **5.5 Applications and Functions in the Cosmetics Industry**

The epidermis (outermost layer of human skin) is covered and protected by a lipid and sweat layer called the acid mantle. The lipid part of the acid mantle is made up of sebum (from sebaceous glands) as well as lipids (from the stratum corneum, the outermost layer of the epidermis). Sweat glands secrete the sweat of the acid mantle. The acid mantle has an acidic pH. With a pH between 4 and 6.5, the skin is protected from bacterial and fungal infections as well as water loss. The acid mantle also supports the barrier function of the stratum corneum. If the acid mantle loses its acidity, the skin will become susceptible to damage and infection, as well as irritation and sensitivity (Shutes, 2020).

The stratum corneum is made up of corneocytes cells and a complex of intercellular lipids that holds moisture. The stratum corneum maintains the water level of the skin below and controls *trans* epidermal water loss (TEWL), which is the natural moisture flow out from deeper skin layers to be lost eventually by evaporation from the skin surface. The cells in the stratum corneum (the corneocytes) form a water-retaining barrier embedded in a lipid matrix (Shutes, 2020).

The lipids that make up the stratum corneum include ceramides (approximately 40–50%), cholesterol (20–25%), and fatty acids (10–25%). These lipids serve to prevent water loss through the stratum corneum. These lipids and the natural moisturizing factor (NMF) of the stratum corneum are important in maintaining the water level of the skin as well as to reduce TEWL. The NMF, which is housed within the corneocytes, is composed of free amino acids and its derivatives, urocanic acid, inorganic salts, sugars, lactic acid, and urea. The NMF components are highly efficient humectants that attract and bind water from the atmosphere, drawing it into the corneocytes. These compounds are also responsible for keeping the skin moist and pliable by attracting and holding water. They can hold large amounts

of water in the skin cells and also capable of absorbing water from the atmosphere and/or products applied to the skin. The lipids serve to prevent water loss from occurring in the NMF (Shutes, 2020).

The fatty acids in the skin lubricate, soften, and protect skin and also prevent moisture loss from the skin. Both essential and non-essential fatty acids play separate and critical roles in proper skin functioning. Two types of essential fatty acids (EFA), linoleic acid and  $\alpha$ -linoleic acid, cannot be synthesized by the body and should be consumed in the diet or applied to the skin. Fatty acids found in the skin include palmitic acid, oleic acid, myristic acid, stearic acid and others. Non-essential fatty acids can be produced by the body, although they can also be ingested or applied to the skin (Shutes, 2020).

A deficiency in stratum corneum lipids may contribute to dehydrated skin or xerosis (abnormal dryness of the skin or mucus membranes). Factors contributing to this condition include age, low humidity, cold and heat exposure (e.g. sunburn, wind burn, or frostbite), diet and genetics. Other factors that can break down the protective lipid layer and increase TEWL include long, hot showers, harsh detergents or solvents, excessive hand washing, and application of irritating chemicals. One way to maintain the health of the skin is to apply vegetable/herbal/nut/seed oils to the skin. These oils, along with creams, lotions, ointments, butters and balms maintain the skin's tone and elasticity, prevent TEWL and support the lipid matrix. Vegetable and seed oils, beeswax, squalene, lanolin and shea butter have a hydrating effect on the skin. They are called occlusive substances and form a barrier on the surface of the skin and help to reduce TEWL. Vegetable oils are used to dilute and carry essential oils onto skin. They can also be therapeutic substances, in that they contain the following chemical components: (1) essential and non-essential fatty acids, (2) fat-soluble vitamins, (3) sterols/phytosterols, and (4) polyphenols/phenolic compounds (Shutes, 2020).

1. Essential and non-essential fatty acids: EFA in vegetable oils help restore the skin barrier and treat inflammatory disorders of the skin such as dermatitis, psoriasis, and eczema. They help wounds heal and assist in the prevention of wrinkles. A deficiency in fatty acids can cause a disruption in the epidermic homeostasis, which affects the barrier function of the skin. This can then lead to TEWL, which can lead to skin disorders such as dryness, scaliness, redness, dermatitis and other signs of inflammation. As mentioned earlier, linoleic acid is one of the two types of EFA. Linoleic acid, the most abundant PUFA, is present in the epidermis. Linoleic acid, an *omega*-6 fatty acid, is an EFA in the skin that required for the formation and maintenance of the cutaneous barrier to water loss. If the water content of the stratum corneum (commonly caused by a breakdown or assault on the skin barrier) falls below 10%, the natural functions are impaired and the skin becomes dry (dehydrated), scaly, and less pliable, all signs of xerosis. Linoleic acid is crucial to the proper growth and development of the epidermis. It is also required for synthesis of the important long-chain ceramides necessary to protect against dry skin. The other EFA in vegetable oils is  $\alpha$ -linoleic acid (ALA). It reduces inflammation when applied topically and can reduce acne (Shutes, 2020).

2. Fat-soluble vitamins: vitamin E, or tocopherols, is a potent antioxidant in vegetable oils. Antioxidants prevent free radicals from causing cell damage. Free radicals damage the collagen (the main component in connective skin tissue) and elastin fibres in the skin. Tocopherols are free radical scavengers. Vitamin E aids to heal, repair, and regenerate skin. There are several types of tocopherols including alpha ( $\alpha$ ), beta ( $\beta$ ), gamma ( $\gamma$ ), and delta ( $\delta$ ) tocopherols. Vegetable oils are also a good source of fat-soluble vitamins A, D and K. When tocopherols are present in a PUFA-rich vegetable oil, the oil's lipids become more stable (Shutes, 2020; de Wit et al., 2021).
3. Sterols/phytosterols: phytosterols are components found in vegetable oils that resemble cholesterol components. Like cholesterol, phytosterols have a water-binding capacity that may help maintain a healthy skin barrier function. When phytosterols are topically applied to the skin, anti-aging benefits may occur. They not only stop the slow-down of collagen production caused by sun damage, but they can also encourage new collagen production (Shutes, 2020). In a study that focused on topical treatments containing phytosterols, it was found that phytosterols not only stopped the slow-down of collagen production, it also encouraged new collagen production. It was, therefore, suggested that phytosterols can reverse the effects of aging and may be useful additions to anti-aging products. The seed oils of *Opuntia ficus indica* and *Opuntia dillenii*'s sterolic fractions composed of  $\beta$ -sitosterol (21.9% and 2.80%, respectively), campesterol (3.75% and 0.51%, respectively), stigmasterol (1.64% and 0%, respectively), and fucosterol (0% and 0.27%, respectively). The main sterol,  $\beta$ -sitosterol, accounted for 80.2% and 78.2% of the total sterol content in *Opuntia ficus indica* and *Opuntia dillenii* seed oils (Shutes, 2020).
4. Polyphenols/phenolic compounds: phenolic compounds are found in vegetable oils as a large class of chemical compounds. They provide the body with antioxidant, anti-inflammatory, anti-carcinogenic and oxidative stress prevention. Phenolic compounds prevent skin damage from sunlight's UV rays and can ameliorate adverse skin reactions following UV exposure including skin damage, erythema (redness or rash resulting from capillary congestion), and lipid peroxidation (Shutes, 2020).

In a study by Ciriminna et al. (2017), the cosmetic and nutraceutical applications of *O. ficus-indica* and *O. dillenii* were evaluated. The authors reported the oils' anti-oxidative and hydration action on skin and hair, imparting cosmetic properties, while the UFA impart anti-inflammatory properties, showing significant potential to also be used as functional ingredients in nutraceuticals and food supplements. Cosmetic vegetable oils have moisturizing properties and valued because of their characteristic dermatological properties. *Opuntia ficus-indica* seed oil's specific chemical composition makes it a valuable and ideal ingredient in cosmetic applications (Gharby et al., 2020). In a study by Regalado-Rentería et al. (2018), application of cactus pear seed oil prevented the *in vitro* growth of human skin fibroblasts cells during radiation. Radiation caused oxidative stress, leading to the production

of free radicals. These were neutralized by the antioxidants in the oil, wherein UV light can be absorbed by the PUFA. Cactus pear seed oil could, therefore, be used in sunscreens. Tunisian prickly pear oil absorbed UV-A, UV-B as well as UV-C radiation.

## 5.6 Summary of Food and Non-Food Applications of Cactus Pear Seed Oil

A summary of the food and non-food applications of seed oil from various *Opuntia* spp. are indicated in Table 48.1.

**Table 48.1** Food and non-food applications of *Opuntia* spp. seed oil

Specie	Industry	Function	Responsible compound	Reference
<i>O. dillenii</i>	Food	Fat replacer	NR	Koubaa et al. (2016)
<i>O. dillenii</i>	Food	Antioxidant	FA	Ali-Alsaad et al. (2019)
<i>O. ficus-indica</i>	Food	Antioxidant, Antimicrobial	Polyphenols, Tannins, and flavonoids	Brahmi et al. (2020)
<i>O. ficus-indica</i> , <i>O. albicarpa</i>	Food, medicinal	Antimicrobial, Antifungal	NR	Ramírez-Moreno et al. (2017)
<i>O. ficus-indica</i> , <i>O. albicarpa</i>	Food, medicinal	Antimicrobial; more effective against gram positive than gram negative	NR	De los Angeles Ortega-Ortega et al. (2017)
<i>O. ficus-indica</i> , <i>O. albicarpa</i>	Food, medicinal	Anti-microbial against <i>Enterobacter cloacae</i> (bacteriostatic and bacteriocidal) Antifungal against <i>Candida</i> spp.	Extra-virgin cold pressed UFA, triacylglycerols, phytosterols, tocopherols	Khémiri et al. (2019)
<i>O. ficus-indica</i>	Medicinal	Anti-cancer	Seed oil	Sreekanth et al. (2007)
<i>O. ficus-indica</i>	Medicinal	Anti-hyperglycaemic, prevent alloxan-induced diabetes, acarbose inhibit $\alpha$ -glucosidase	NR	Berraouan et al. (2014)

(continued)

**Table 48.1** (continued)

Specie	Industry	Function	Responsible compound	Reference
<i>O. ficus-indica</i>	Medicinal	Anti-inflammatory	FA	Koshak et al. (2020)
<i>O. dillenii</i>	Medicinal	Antioxidant	Seed oil	Chang et al. (2008) and Liu et al. (2009)
<i>O. dillenii</i>	Medicinal	Lower cholesterol	Phytosterols lead to lower absorption of cholesterol	Jiménez-Aguilar et al. (2015)
<i>O. dillenii</i> , <i>O. matudae</i>	Medicinal	Antioxidant, Anti-inflammatory, anti-allergic, Anti-atherogenic, cardio-protective	– Phenols (phenolic acids, flavonoids, hydrobenzoic acids) – Seeds (phenolics and flavonoids)	Chang et al. (2008)
<i>Opuntia</i>	Medicinal	Various	Various plant parts	Nazareno (2015)
<i>O. ficus-indica</i> , <i>O. megacantha</i> , <i>O. albicarpa</i> , <i>O. streptacantha</i> , <i>O. robusta</i> , <i>O. matudae</i>	Medicinal/ cosmetic	Absorbs UV radiation	UFA	Regalado-Rentería et al. (2018)
<i>O. dillenii</i>	Cosmetic	Essential oils	NR	Koubaa et al. (2016)
<i>O. dillenii</i>	Cosmetic	Anti-ageing, Anti-wrinkle	Organic oil	Koubaa et al. (2016)
<i>O. dillenii</i>	Pharmaceutical	Essential oils	Vitamin homologues isoforms ( $\gamma$ and $\beta$ tocopherols)	Koubaa et al. (2016)
<i>O. ficus-indica</i>	Agronomic attract <i>Ceratitidis capitata</i> pest	Antioxidant, Antimicrobial	Hexadecanoic acid/kairomones	Zito et al. (2013)
<i>O. ficus-barbarica</i>			41 compounds	Al Juhaimi et al. (2020)
<i>O. macrorrhiza</i> <i>O. microdasys</i>		Antioxidant		Chahdoura et al. (2015)
<i>Opuntia</i> spp	Food/medicinal	Bioactive Phytochemicals	Bioactive compounds	Barba et al. (2017)
<i>Opuntia ficus-indica</i>	Food/medicinal	Antioxidant, Stability	Cold pressed oil, Fatty acids, tocopherols	de Wit et al. (2020, 2021)

NR not reported

## 6 Conclusion

Cactus pear seed oil is gaining increased interest for its health benefits and its popularity in the nutraceutical, cosmetics and food industries. With large amounts of linoleic acid, vitamin E, phytosterols and phenolics, cactus pear seed oil and its components make the oil an extremely rich and skin-nourishing oil. It prevents and cure many diseases and has excellent antioxidant and antimicrobial properties. Cactus pear seed oil's cost is worth every cent.

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# Chapter 49

## *Opuntia* spp. in the Textile Industry



Ahmed A. Hussein and Fei Wang

**Abstract** The textile industry is one of the anthropogenic activities that most consume/pollute drinking water. Some of the synthetic dyes are highly toxic, mutagenic, and causing oxygen debilitation in water. The use of biocompatible chemicals started to be increased substantially over the last few years for its safety and health benefits to reduce environmental pollution. The use of eco-friendly dyes for textiles is highly appreciated because of the sustainability, biocompatibility, biodegradability, and various therapeutic advantage such as antibacterial, antifungal, and antioxidant properties. Plants belonging to the *Opuntia* genus are important natural sources that contain important natural dyes that can be used in the textile industry. The fruit contains dyes that belong to betalain skeletons and are responsible for yellow and red colours. These pigments can be used as a natural and safe alternative to synthetic colours. This chapter discusses the potential of *Opuntia* fruits' different constituents in the textile industry.

**Keywords** Natural colour · Indicaxanthins · Betanin · Textile · Antibacterial

### 1 Introduction

The textile industry is one of the biggest industries, globally worth over \$450 billion, in terms of nominal sales (Euler Hermes Economic Research, 2016). It is one of the most jobs creating industries in the world, contributed nearly 7% of world production among all manufacturing sectors globally (Allwood et al., 2006; Herva

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et al., 2008; Desore & Narula, 2018). On the other hand, it is regarded as one of the biggest global polluters (De Brito et al., 2008; Hossain et al., 2018).

The production processes intensively use chemicals and water, which generate a high environmental negative impact. The chemical compounds such as dyes are partially utilized and washed away with the effluents (Islam & Khan, 2014). The non-biodegradable and carcinogenic components of these chemicals directly contaminate the water resources and affect the biological system (Hessel et al., 2007). Over 10,000 dyes and pigments are used in different industrial sectors, including textiles. The resulted washing of the dyeing process produces a large volume of liquid effluents contain (10–60%) the unreacted portions of these dyes and pigments with other chemical products and auxiliaries such as phosphates, nitrates that affect aquatic fauna, flora and human health (Berradi et al., 2019).

During the last years, the demand for environmental-friendly textiles and clothes, manufactured and distributed, minimizing the negative impacts of the toxic chemicals on the environment, has vigorously emerged from many stakeholders, including consumers (Lellis et al., 2019). Subsequently, steps have been implemented to make textile materials and processing more environmentally friendly, including dyes and their applications (Roy Choudhury, 2013). Green chemistry has emerged as a useful tool to make textile wet processing sustainable and develop alternative green and biodegradable chemicals such as biodegradable natural dyes (Gulzar et al., 2019).

## 2 Natural Dyes from *Opuntia*

Color is the main attraction of any fabric. The use of natural dyes for fabric dyeing has been known to humankind since immemorial times. Natural dyeing was found very encouraging, with its non-toxic, non-allergic, and non-carcinogenic soothing harmless effects. Moreover, these dyes are cost-effective and eco-friendly. Natural dyes work on cotton, silk, and wool. Their colours are relatively stable and eco-friendly because of no irritating effects on human skin. Different plant such as madder, walnut, weld, white onion, red onion, pomegranate, turmeric, barberry, and cactus have been reported as a source for natural dyes (Kasiri & Safapour, 2015).

The prickly pear or cactus pear are general names for different *Opuntia* species. It belongs to the Cactaceae family, which is native to the new world. Some species, mostly *O. ficus indica* cultivated in different regions of the world. The red pigments in *Opuntia*, known as betalains pigments, have a different range of colours, such as red-violet (betacyanins) and yellow-orange (betaxanthins) (Scarano et al., 2020). The relative proportions of betacyanins and betaxanthins are responsible for the enormous range of colors of the flowers of bougainvillea vines. There are 75 compounds described so far in the literature, 17 of them identified in different *Opuntia* species. However, betanin and indicaxanthin are the most common dyes distributed among *Opuntia* species (Khan & Giridhar, 2015; Check Aruwa et al., 2018).

Betaxanthins (Latin: beta = beet; Greek: xanthos = yellow) are condensation products of betalamic acid and amino acids or amines, respectively. Depending on the amino compound's particular structure, the maximum absorption of betaxanthins varies between 460 and 480 nm (Stintzing et al., 2002). Indicaxanthin is the most common and predominant pigment in yellow cactus pears (*Opuntia* spp.). Betaxanthins-related structures are more diverse than the betacyanins (Castellanos-Santiago & Yahia, 2008; Cejudo-Bastante et al., 2014; Aruwa et al., 2018). In addition to visible variable colors range from yellow to reddish violet, betalains also showed interesting fluorescent properties. Most betaxanthins are weakly fluorescent in aqueous solution and are responsible for the fluorescence of the yellow flowers of *Mirabilis jalapa* and *Portulaca grandiflora*. On the other hand, betacyanins are non-fluorescent (Gonçalves et al., 2015). The discovery of the fluorescence phenomenon of betalains' in 2005 (Gandía-Herrero et al., 2005) opens up new applications in different fields, including the textile industry (Guesmi et al., 2012a). Metal cations can bind to betalains *via* complexation with the 1,2,3,4-tetrahydropyridine 2,6-dicarboxylic acid moiety of the betalamic acid portion molecule. The complex formation between betalain with  $\text{Eu}^{3+}$  forms a stable orange-color (Quina & Bastos, 2018).

Betalains are chemically labile in highly acidic aqueous media ( $\text{pH} < 1$ ), and alkaline hydrolysis occurs at  $\text{pH} > 8$ , restricting most studies in aqueous media to slightly acidic conditions ( $4 < \text{pH} < 6$ ). Even at  $\text{pH} 5$ , most derivatives' thermal decomposition occurs in a few minutes at  $100\text{ }^\circ\text{C}$  (Herbach et al., 2006a, b). Betalains are also subject to metal-ion catalyzed decomposition (Belhadji et al., 2017).

### 3 Dye extraction

Betalains are hydrophilic compounds and can be easily extracted using aqueous-alcoholic solutions or water. Due to the stability issues, usually adjusting the  $\text{pH}$  to acidic media and/or antioxidants like ascorbic acid be added during the extraction procedures. Ultrasonic-aided extraction was applied to improve the dye's extraction (Sivakumar et al., 2009; Guesmi et al., 2012b, 2013a, c; Yaqub et al., 2018; Salem et al., 2020). Highly pure betanin was extracted using ultrasonic from the reddish-violet fruit homogenate using water followed by fractional crystallization (Guesmi et al., 2013c). From the orange-yellow fruits, yellow pigments (indicaxanthin) was extracted with 80% aqueous ethanol, the partial purification completed using acidified C18 cartridge and ethanol as eluent (Guesmi et al., 2012a, 2013a). Acidified water was also used to enhance the dye stability during the extraction process (Guesmi et al., 2012b; Salem et al., 2020). Comparing the maceration extraction with the Naviglio extraction method, under the same extraction conditions (solvent, time,  $\text{pH}$ , ratio sample: solvent), the measurements indicated that the Naviglio method had higher dye content a short time. The extractor Naviglio® is a new solid-liquid extraction technology based on the suction effect (Salem et al., 2020).

Supercritical fluid extraction (SFE) with CO<sub>2</sub> as a solvent and EtOH/water as co-solvent was used and optimized for the extraction of betacyanins from red pitaya fruit (*Hylocereus polyrhizus*) peel. At optimal condition, the extraction yield of 4.09%, total betacyanins content of 25.5 mg/100 mL was recovered (Fathordoobady et al., 2019; Luo et al., 2014).

Antioxidants compounds such as ascorbic and isoascorbic acids as antioxidant agents and citric acid as a chelating agent were reported to enhance betalain stability and prevent degradation during the thermal process (Attoe & Von Elbe, 1982; Han et al., 1998; Herbach et al., 2006a, b; Wendel et al., 2015). EDTA was proven to increase the half-life time of betanin by 1.5 times (Pasch & Von Elbe, 1979),

## 4 Factors Affecting the Dyeing Process

During the dyeing process, the textile fibers are in continuous contact with all the dye liquor, and therefore the fibers gradually absorb the dyes. To obtain well-penetrated dyeing, some variables such as the dyeing temperature, pH, and the concentration of auxiliary chemicals must be carefully controlled. Different factors affect the quality of dye application to the textile materials, such as pH, temperature, tissue type, salt concentration, and mordants nature.

### 4.1 pH

The presence of nitrogen-containing and carboxylic groups in betalains chemical structures represent proton donor/acceptor centers. Protonation of the nitrogen atoms could occur in an acidic solution. The charge alteration of betanin upon pH changes was proposed (Simon et al., 1993; Filgueiras et al., 2000). The adsorption of the dye is mostly affected by the pH of the dyeing solution. The lower pH could form positive charges on the surface of fabrics such as wool and cause ionic interaction with the dyes' carboxyl groups. On the other hand, the dyes' thermal stability at lower pH may negatively affect the stability of the dyes (Guesmi et al., 2012b, 2013a, c). The optimum pH was recorded between 3.0–5.0. Fabrics such as wool, silk, and acrylics can be directly affected by changing the pH (Savolainen & Kuusi, 1978; Ganesan & Karthik, 2017; Scarano et al., 2020).

### 4.2 Salt Concentration

During the dye diffusion into the textile fibers, an electrostatic charge barrier that develops on the surface of the fibre must be overcome. In some cases, salt in large amounts is added to the dyeing bath to reduce the electrostatic charge on the surface

of the fibre and even promote the dye's penetration. The addition of NaCl to the dyeing bath at different concentrations showed adverse effects. Increasing the salt (NaCl) concentration decreases the color strength of the dyed wool and acrylic fabrics (Guesmi et al., 2012b, 2013a, c).

### **4.3 Temperature**

The dye/fibre interaction depends on many factors that control the dye diffusion into the fibre. The temperature is one of the important factors that control the diffusion process (Schönberger & Schäfer, 2003). Cotton and wool's dyeing process was reported to be effective at 80 °C for 60 min in the presence of mordants (Scarano et al., 2020). On the other hand, the color strength of dyed wool increase as the temperature increases up to 40 °C then it decreases drastically after 50 °C (Guesmi et al., 2013c). In modified acrylics, the optimum temperature of dyeing with betanin was found to be 50 °C (Guesmi et al., 2012b). The result can be a consequence of the losses of betanin stability or dye molecules' desorption from the fibers at high temperatures. The dyeing with pure indicaxanthin showed dye stability and increasing the color strength by increasing the temperature up to 80 °C (Guesmi et al., 2013a).

### **4.4 Ultrasound Irradiation**

The use of ultrasound in textile dyeing showed color strength enhancement and reduced dyeing time (Vankar et al., 2007). The use of ultrasound in dyeing wool with betanin dye demonstrated that using a lower frequency of ultrasound irradiation increases dye exhaustion (25 kHz > 40 kHz > conventional heating) till 30 min and then it decreases slowly (Guesmi et al., 2013c, b). The ultrasound assisted in uniform dispersions in the dye on the fabrics and resulted in a higher depth shade (Ghorpade et al., 2000). The dyeing of the modified acrylic fiber with ultrasonic also improved indicaxanthin dye uptake (Guesmi et al., 2013a).

### **4.5 Effect of Bio/Mordant**

The use of natural dyes has always been bounded to the use of the metallic salts as mordants to improve fastness properties (Cristea & Vilarem, 2006). Dyeing of fabrics with natural dyes often leads to problems such as narrow shade range and lower colorfastness of the dyed textiles. Attempts to overcome these problems have been mainly focused on the use of mordant. The term "mordant" is often used for chemicals, including metal with at least two or more valence that increases affinity



between the dye and the fiber and is also used to change the hue of dyes (Shahid & Mohammad, 2013). The mordants' main function in textiles is to fix a dye to the fibers provide and obtain different colors. They also improve the take-up quality of the fabric and help improve color and light-fastness. In the mordanting process, firstly, the fabric is impregnated with the mordant, and then the dye reacts with the mordant for forming a chemical bond and attaching it firmly to the fabric during the dyeing process (Abuamer et al., 2014). The salts of di- and trivalent metals are the most common mordants, however in the last few years, biomordants such as chlorophyll a (Guesmi et al., 2013c) are tannic citric, tartaric, and acetic acids have been reported (Yaqub et al., 2018).

Different types of mordents such as potassium dichromate, copper sulphate, ferrous sulphate, and tannic acid were used as mordants during dyeing wool with an aqueous extract of red prickly pear fruits. The use of potassium dichromate and tannic acid improved the lightfastness of wool dyed with betalains pigments more than copper sulphate and ferrous sulphate. On the other hand, using tannic acid improved the washing fastness than other mordents. Also, the post-mordenting gives maximum colour strength when compared with pre-mordenting (Ali & El-Mohamedy, 2011).

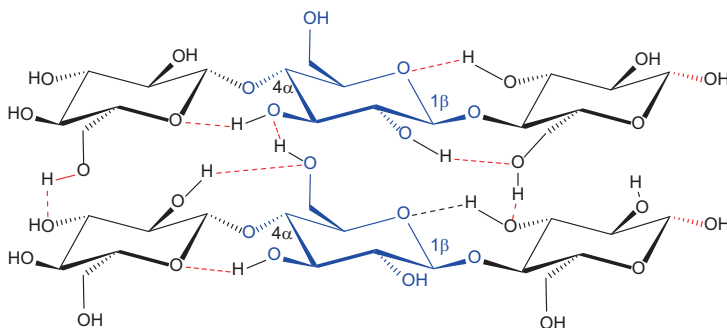
The metallic mordant concentration has a negative effect on the colour strength (Ali & El-Mohamedy, 2011). This was supported by the results that showed metal cations ( $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Sn}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cu}^{2+}$ ) accelerate betanin degradation (Attoe & Joachim, 1984). On the other hand, increasing the concentration of biomordant such as chlorophyll a was found to enhance the dyeability due to the release of Mg cation liberated at acidic pH (Diop Ndiaye et al., 2011; Guesmi et al., 2013c).

## 5 Natural Fibers

### 5.1 Cotton

Cotton fibers are the purest form of cellulose. The hydrophilic nature of the cellulose fibers is reflected from the abundant hydroxyl groups, which allow extensive intermolecular and intramolecular hydrogen bonding (Fig. 49.1), which result in various ordered crystalline arrangements. These hydroxyl groups are also critical for the chemical interaction with dyes (Hsieh, 2007; Park et al., 2010). The cellulose fibers usually exist in crystalline form. However, the amorphous structure also present. The diffusion of the dyes through the crystalline cellulose is difficult, and the final dyeing results depend on the amount of the amorphous phase present.

Different natural dyes such as Madder (*Rubia tinctorum*), weld (*Reseda luteola*), and coreopsis (*Coreopsis tinctoria*), have been used to dye cotton (Haar et al., 2013). The application of natural dyes to cotton usually associated with poor fastness (Cristea & Vilarem, 2006), and the use of mordant such as aluminum acetate, copper sulfate, and potassium dichromate are highly recommended (Haar et al., 2013).



**Fig. 49.1** Cellulose linear structure shows inter- and intra-hydrogen bonding (dashed line)

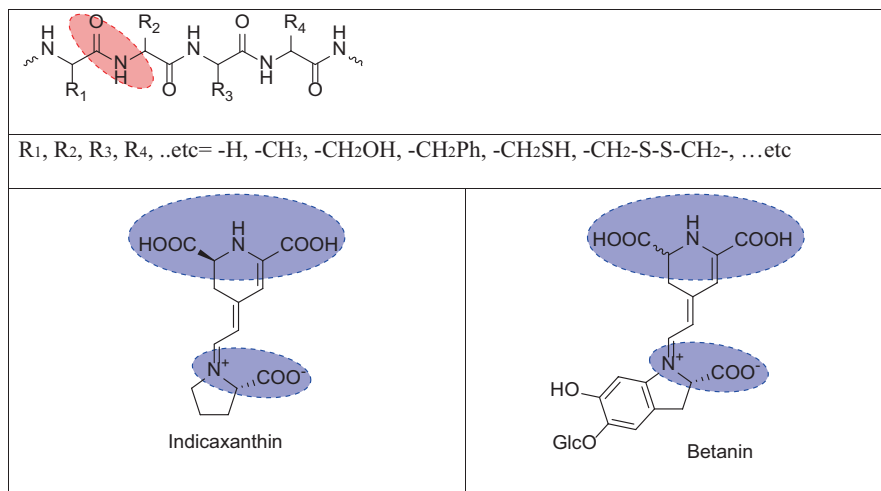
The green synthesis strategy was established for textile finishing, aiming at the development of antimicrobial-printed cotton. A natural dye extract obtained from red prickly pears was applied with or without mordants (tannic acid) to chitosan-treated cotton. The result that cotton fabrics containing chitosan had higher color strength than untreated fabrics with or without the mordant. The printed fibres also showed good antibacterial activity (Hebeish et al., 2011, 2012).

## 5.2 Wool

Wool has a typical protein structure build-up from amino acids linked together through peptide bonds. Parts of the protein chain exist in crystalline form and have an  $\alpha$ -helical structure stabilized by intramolecular hydrogen bonding between NH and CO groups. The similarity in chemical structures between the wool and natural dyes from *Opuntia* proposes strong intermolecular hydrogen bonding and electrostatic interactions, and these are reflected from the presence of donor and acceptor active groups among each structure (Fig. 49.2). The dye's affinity to the wool fabrics is expected to be good and can be improved using temperature, sonication, mordant and low pH.

The colour strength and the dye uptake by the wool fabrics have exhibited high values. Good fastness properties of the dyed fabric were achieved using different mordants. This depends on the groups capable of forming hydrogen bonding and a metal complex. The higher the number of these groups, the lower the magnitude of the dye removal. Thus, mordanting with tannic acid gave the highest washing fastness, followed by potassium dichromate. The dyeing process's optimum conditions were observed at pH 5 and temperature 100 °C (Ali & El-Mohamedy, 2011).

Rubbing fastness was high in the mordanted samples. The lightfastness of samples mordanted with potassium dichromate and tannic acid was found to be more than samples mordanted by copper sulphate and ferrous sulphate comparing, while the unmordanted samples showed inferior fastness (Ali & El-Mohamedy, 2011).



**Fig. 49.2** Active chemical sites in indicaxanthin and betanin

Antimicrobial wool fabric activity showed a high inhibition zone at 2% of the dye concentration against *Bacillus subtilus* and *E. coli* (Ali & El-Mohamedy, 2011).

A stable color was achieved by the addition of ascorbic acid and different mordants. The dry cleaning fastness was excellent, while the wash fastness in neutral detergent was more effective than alkaline detergent. The perspiration fastness was increased by the mordanting method. On the other hand, the lightfastness was inferior. The *Opuntia* extracted dye improved the antibacterial and UV-B protection properties of wool fabrics (Lee et al., 2006).

Due to the natural variation of natural products among natural resources, the dyeing with total extracted dyes showed a reproducibility problem (Türkmen et al., 2004) using single pure compounds such as betanin or indicaxanthin is preferable. The dyeing of wool fabric using purified betanin as natural dye and chlorophyll a as biomordant showed an increase in the colour strength values with increasing the concentration of biomordant. The depth of shade reached its maximum value at 45 min. On the other hand, the optimum temperature for dyeing was found to be 40 °C, and the optimum pH was 4.5, at acidic conditions, the Mg is released from the porphyrin ring of chlorophyll a and enhance the dyeability of the betanin. The fastness properties of dyed wool against washing, light, dry and wet rubbing showed an improvement. The mordant samples showed good fastness properties than the un-mordanted ones (Guesmi et al., 2013c). The same group studied the dyeing of wool with indicaxanthin. The dye's adsorption on the wool was found to be optimum at pH 4 and temperature between 70–80 °C. The use of mordant directly affected the dyeing process. The colour strength increased in the order of the dyeing using  $\text{KAl}(\text{SO}_4)_2 > \text{MnSO}_4 > \text{CoSO}_4 > \text{FeSO}_4 > \text{none} > \text{ZnSO}_4 > \text{CuSO}_4$ . Un-mordanted samples have good properties of water and washing fastness.

Mordants  $KAl(SO_4)_2$  and  $CoSO_4$  were found to give good lightfastness (Guesmi et al., 2012a).

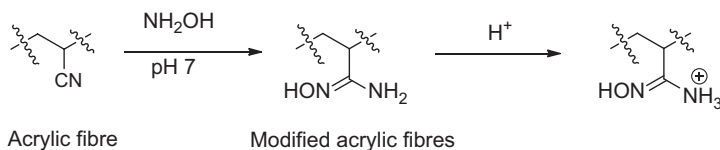
The wool fabric dyed with indicaxanthin showed interesting fluorescence properties. Photoluminescence was recorded at 576 nm. The samples showed a weak response to light irradiation but open up a new field of the corporation of natural fluorescent dyes into textile fabrics (Guesmi et al., 2013d).

### 5.3 Silk

Fibroin is the structural protein of silk fiber; it has a typical protein structure where amino acids are linked together by peptide bonds. The presence of active carboxylic and amino groups enhances the interaction with dyes and improves the fastness. The aqueous extract of the red prickly pear fruit was used to dye silk fabric. Myrobalan is a rich tannin extract used as a biomordant and copper sulphate (Ashrafi et al., 2018; Jain & Vasantha, 2016). The post-mordant-processed fabric with  $CuSO_4$  showed higher colour strength when compared to myrobalan. The superiority of  $CuSO_4$  in improving the dyeing process may reflect the metal complex's importance than tannin effects. The antibacterial properties of dyed fabric without mordant showed better results than pre-mordant and post-mordant samples, and the antibacterial activity decreases gradually during further washing cycles (Ganesan & Karthik, 2017). Treatment of Silk fabric with chitosan improved *Opuntia* dye interaction and displayed a good colour strength and high antibacterial activity (Julia & Pascual, 2000; Hebeish et al., 2012).

## 6 Synthetic Fiber

Acrylic fiber is a synthetic polymer composed mainly of acrylonitrile unit. It has a strong hydrophobic character and low dye affinity. Pre-treatment with hydroxylamine hydrochloride (Fig. 49.3) was proposed to improve the fibers' dye-ability (El-Shishtawy & Ahmed, 2005). The modified acrylic fibers were subjected to dyeing with indicaxanthin and betanin dyes purified from *Opuntia* species (Guesmi et al., 2012b, 2013a). The optimal conditions for dyeing with indicaxanthin dye were found at 80 °C for 30 min and pH 3. Sonicator improved the dye uptake and



**Fig. 49.3** Introducing active sites in acrylic fibres by reaction with hydroxylamine

color strength and gave a higher depth shade at a lower dyeing temperature (Guesmi et al., 2013a). On the other hand, the optimal conditions for betanin dye were found at 50 °C for 45 min and pH 5. Using CoSO<sub>4</sub> was found to give good lightfastness. Nevertheless, the un-mordanted samples showed good properties of water and washing fastness (Guesmi et al., 2012b).

## 7 Functional Textiles

Cellulose and protein derived natural fibres provide suitable conditions for bacterial growth and multiplication and result in unpleasant consequences such as foul odors, mold and mildew strains, discoloration, and loss of functional properties (Hebeish et al., 2011). The control of microorganisms' growth on textile fabrics using non-toxic chemicals, environmentally benign and renewable materials is one of the key issues that merit important consideration of the green synthesis strategy (Singh et al., 2005). The use of natural dyes is an important source of antibacterial agents and can play dual roles.

Dyeing of wool fabric with prickly pear 2% dye showed antibacterial and antifungal activities, the highest inhibition zones of 27.9, 18.3, 15.0, and 13.5 mm against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* were recorded (Ali & El-Mohamedy, 2011). The antibacterial activity of silk fabrics dyed with betalains from *Opuntia* showed antimicrobial activity even after 15 washes against *S. aureus* and *E. coli* (Ganesan & Karthik, 2017). Dyes from *Opuntia* were also used for printing cotton and silk that were pretreated with chitosan and tannic acid. The results showed a high growth reduction of microbes, and the growth of *S. aureus* and *E. coli* was inhibited completely (Hebeish et al., 2011).

## 8 Conclusion

In addition to high safety margins, affordability, and availability, natural dyes have multifunctionality, i.e., UV-protection and antibacterial activity. Total extract of *Opuntia* dyes and purified constituents such as betanin and indicaxanthin dyes were found to compete with synthetic dyes in color depth shade and fastness properties. The chemical structure of betalains is unique, where carboxylic and amino functional groups are present. This feature gives an advantage of strong and direct interaction with textile fibers. The dyeing with *Opuntia* dyes can be improved using different factors such as pH, ultrasonic, temperature, and bio/mordants, proper adjustments of these factors enhance the dyeability and fastness of the dyes on the different type of textiles. The nature of the natural dyes' chemical structures and stability is one of the major drawbacks reflected from the poor wash and light fastness of textile fibers. More research will be highly appreciated in this regard,

considering the importance of using a standardized total extract. Although the industrial application of natural dyes, including *Opuntia*, is not among the major textile industry, makers' scc lightfastness scale and handmade industry look for more safe and affordable natural dyes.

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# Chapter 50

## Cactus Pear as Colorants and Coloring Foods: Application in Different Food Matrices



Carmen Sáenz, Juan Carlos Carmona, Cristina Vergara, and Paz Robert

**Abstract** Food color is one of the most important attributes related to consumer acceptance. The growth in consumer trends for the preference of healthy food colorants, clean-label foods, and attractive appearance, among other factors, has driven the global market of natural colorants to rise in recent years. Cactus pear fruits (*Opuntia* sp.) are a good source of betalains, which are natural pigments that cover a wide range of colors: from red-purple betacyanins to yellow-orange betaxanthins, with each species possessing different pigments and contents. Betalains are water-soluble pigments and could replace artificial colors in food, many of which have been questioned as harmful for health. The extraction, stability, and application of pigments has recently received particular attention due to their appeal as a natural ingredient. Furthermore, they come from a species with a low water requirement, which is increasingly appreciated in the face of climate change that is affecting the planet. This chapter analyses the variation of betalain content according to the *Opuntia* species and the different types of colorants obtained from colored cactus pears, concentrated pulp and juices, coloring foods (a new concept introduced by the EU legislation), powders, microparticles, among others. The stability of betalains, a critical factor for their successful use in foods, depends both on the form the pigment is applied (i.e., as concentrated juice or microencapsulated) and on the food matrix in which it is applied. Although there are still many food matrices in which their application has not been studied there are forms of colorants that are sufficiently

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stable to be used in a variety of products. They are more stable in yogurt type matrices than in soft drink type matrices, with yellow-orange pigments also showing more stability than red-purple ones. Thus, a new source of natural pigments is making its way into a market that increasingly values healthy and environmentally friendly ingredients.

**Keywords** Food colorants · Coloring foods · Betalains · Betacyanins · Betaxanthins · Pigment application in foods

## 1 Cactus Pear as a Source of Colorants for Foods

Color is the single most important quality attribute that affects the consumer's acceptance of foods since it provides the first impression of quality. Colorants are widely used in the food industry to make foods more attractive or to restore the original color after processing and to ensure quality. The global market for natural food colorants will grow to USD 1657.7 million by the end of 2023 (Researchester.com, 2020). In this context, there is a worldwide trend toward the use of natural food colorants. Betalains are one of the few red-purple and yellow-orange natural water-soluble pigments, which makes them suitable for use as colorants in many different food products (Azeredo, 2009; Castellar et al., 2003). Their stability is higher at pH 3–7, both in pure solutions and in extracts and juices (Castellar et al., 2003; Moßhammer et al., 2005a; Stintzing & Carle, 2004). Therefore, the cactus pear is a promising source of food colorants to add to acidic products such as yogurt, ice cream, and beverages (Buchweitz et al., 2013; Herbach et al., 2006).

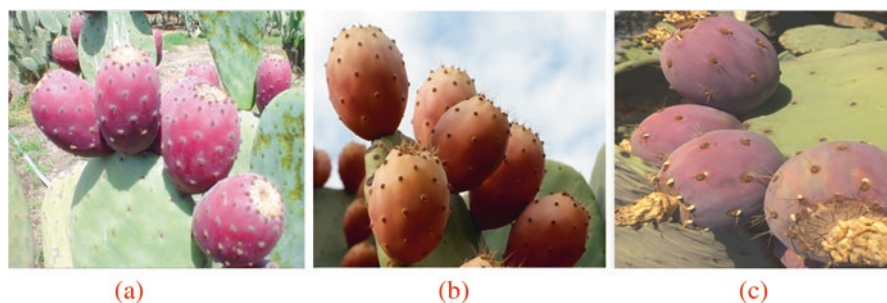
Red-purple betalains are commercially extracted from the red beet (*Beta vulgaris*), but the cactus pear, as a fruit, unlike the beet, lacks compounds such as geosmin and pyrazine, which deliver flavors not desired by consumers (Kaimainen et al., 2015; Vergara et al., 2014). However, yellow-orange betalains are not currently being extracted from any raw material for commercial use. Besides betalains, cactus pear fruits also have other bioactive compounds such as vitamin C and polyphenols (Obón et al., 2009; Sáenz et al., 2012).

Betalains are betalamic acid derivatives and classified into two groups: the red-violet betacyanins (iminium adduct of betalamic acid and *cyclo*-DOPA) and the yellow-orange betaxanthins (betalamic acid condensed with an amino acid). The main betacyanin is betanin, and the main betaxanthin is indicaxanthin (proline-betaxanthin) (Delgado-Vargas et al., 2000; Gandía-Herrero et al., 2012). Betanin, also called “beetroot-red”, is accepted for use in food and among natural pigments being classified as additive E-162 (EU) and 73.40 (FDA, USA). It is conceivable that if both compounds have similar chemical compositions and come from an edible fruit, that the betaxanthins could become a commercial additive, and receive the

approval of international regulatory bodies. Purple, red, or orange cactus pear fruits of different species and cultivars provide a source of betalains that has been explored less (Fig. 50.1). The fruit's betalain content is affected by several factors such as cultivar, distribution in the fruit (peel or pulp), maturity stage (García-Gutiérrez et al., 2006), and climate or geographical production location (Stintzing & Carle, 2004). Therefore, considering the commercial production of these colorants, it is feasible to select the type of *Opuntia* which will be used as the raw material based on the pigment content. The presence of pigments in both pulp and skin (Odoux & Domínguez López, 1996) increases the usefulness and yield of pigments per fruit.

Betacyanins and betaxanthins have been found in different cultivars of cactus pear and in different contents by Stintzing et al. (2005) for purple, red, and orange juice of *Opuntia ficus-indica* clones with 431.0, 120.0, and 6.6 mg L<sup>-1</sup> of betanin equivalents, and 195.8, 67.9, and 76.3 mg L<sup>-1</sup> of indicaxanthin equivalents, respectively. Fernández-López and Almela (2001) reported 250 mg kg<sup>-1</sup> betaxanthins in the fresh pulp of a yellow *Opuntia ficus-indica* cultivar. Morales et al. (2009) reported 111.0 and 29.3 mg kg<sup>-1</sup> of betacyanin and 2.1 and 89.4 mg kg<sup>-1</sup> of betaxanthin, in orange and purple cactus pear pulp (*Opuntia ficus-indica*), respectively. Butera et al. (2002) reported 8.4, 2.6, and 5.86 mg 100 g<sup>-1</sup> indicaxanthin in edible pulp and 1.0, 5.1, and 0.1 mg 100 g<sup>-1</sup> of betanin, in yellow, red, and white Sicilian *Opuntia ficus-indica* cultivars, respectively. Carmona et al. (2019) reported values of 82.9 mg kg<sup>-1</sup> in edible pulp in a yellow cultivar (*Opuntia ficus-indica*).

Castellanos-Santiago and Yahia (2008) studied the betalain content in different Mexican cultivars where the highest values of betacyanins were found in the fruit of the *Opuntia robusta* (5.3 mg g<sup>-1</sup>), which is comparable to that found in some red beets (5.4 mg g<sup>-1</sup>). The same study reported high betaxanthin content in yellow/orange cultivars of *Opuntia megacantha* (0.16 mg g<sup>-1</sup> d.w.) and *Opuntia albicarpa* (0.12 mg g<sup>-1</sup> d.w.). Navarro (2018) reported 290 mg kg<sup>-1</sup> of betacyanins in *Opuntia robusta* purple fruits, similar to that reported by Chavez-Santoscoy et al. (2009) for *O. robusta* of 300 µg g<sup>-1</sup>. The content of betaxanthins found in yellow *Opuntia* spp. pulp fruit was 109.5 mg kg<sup>-1</sup> (Monroy-Gutiérrez et al., 2017), which contrasts with what was reported by Chavez-Santoscoy et al. (2009) for the *Opuntia* spp. Pelon

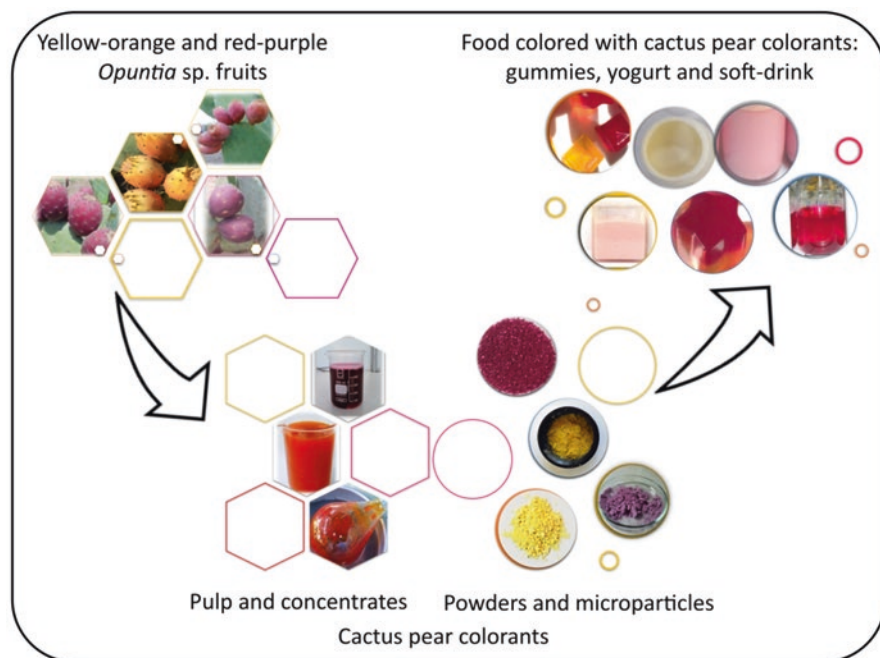


**Fig. 50.1** Different species and cultivars of cactus pear as a source of betalains (a) *Opuntia ficus-indica* (purple), (b) *Opuntia ficus-indica* (yellow-orange), and (c) *Opuntia robusta* (purple). (Photos from the Antumapu Experimental Station, University of Chile (Chile))

cultivar with values between  $46 \mu\text{g g}^{-1}$  and as indicated by Coria Cayupán et al. (2011) for *Opuntia megacantha* of  $52 \mu\text{g g}^{-1}$ . As can be seen, different cultivars have different classes and amounts of betalains (yellow or purple). Therefore, agronomists are faced with a challenge: to improve the pigment content of cultivars to produce colorants.

In this context, a significant advantage for the use of the *Opuntia* fruit for pigment production is that it is a plant species with a low water requirement. Therefore, it can be grown in arid and semi-arid regions, where it is very difficult to cultivate other crops. Starting as a native plant from Mexico, it has spread to many other areas of the world such as Africa, Europe, Asia, and both North and South America (Ochoa & Barbera, 2017).

To summarize, the cactus pear is a source of natural colorant that is viable for developing many agriculturally depressed areas as well as being a very interesting crop for facing climate change. The following will be a discussion of the studies done on the applications of betalains as a colorant in different food matrices, its potential, and its limits. Figure 50.2 shows a graphic diagram of cactus pear colorants, their sources, and applications.



**Fig. 50.2** Graphic diagram of cactus pear colorants, their sources, and applications

## 2 Coloring Foods

In recent years, the coloring food concept has been developed mainly in European countries. The European Commission (EC) has published a guide for classifying food and its extracts as colorants (food additives), food with colorant properties, or coloring foods (not food additives). As a result, coloring foods may not be declared as a food additive, but as an additional ingredient (Bratinova, 2015; Collins, 2014; EC, 2013; Matulka & Tardy, 2014).

For a product to be considered as a coloring food in the European Union, it must be a food normally consumed as such, or as a characteristic ingredient of the food. Furthermore, in the case of extracts, to be considered within this category, when going through the extraction process (physical or chemical) they must not lose the rest of the characteristics (nutritional, aromatic) of the food they come from (EC, 2013). If food or extract is classified as a coloring food, it should not be declared as an additive in the list of ingredients (Bratinova, 2015; Collins, 2014; EC, 2008, 2011, 2013).

This concept is similar to that of the FDA (USA) related to color additives exempt from certification and permanently listed for food use. Among them are dehydrated beets (beet powder) or, in general, vegetable and fruit juices or extracts, used according to Good Manufacturing Practices (GMP)(FDA, 2020).

In this context, fruit juices and pulps are particularly interesting, since they not only provide colors from natural sources but can also be considered as coloring foods, with special health benefits for consumers since they are applied to foods directly, with all their components, including bioactive compounds (Kantha, 2004; Matulka & Tardy, 2014).

## 3 Cactus Pear Products as Colorants for Foods

### 3.1 Cactus Pear Juice and Concentrates

One of the first attempts to use cactus pear as a coloring food was developed by Sáenz et al. (2000) with the purple cactus pear fruit (*O. ficus-indica*). In another study, taking advantage of the fact that the pigments are found both in the fruit pulp and the skin, as noted above, Sáenz et al. (2002) the whole fruit was used, which was pressed to extract the juice. After, the filter cake was extracted twice with water to increase the pigment yield (Sáenz et al., 2012). Then the juice was concentrated, and the betanin content registered 123 mg 100 g<sup>-1</sup>. This product could be a potential natural colorant for food reviewed in previous stability studies at different temperatures. However, depending on the amount of the product used to color food, the sugar content could limit the application to sweet types of food only. In this sense, other technologies will be discussed below.

Moßhammer et al. (2005b) developed a process to produce clarified juice from the pulp of *Opuntia ficus-indica* cv. Gialla and Rossa. Different enzyme preparations were tested to degrade pectic-like substances and improve the filtration process. The resulting juice was pasteurized and had a betacyanin content of 36 mg L<sup>-1</sup> for the cv. Rossa and a betaxanthin content of 45 mg L<sup>-1</sup> for the cv. Gialla juice. Clarified cactus pear juice showed an attractive visual appearance, and the authors concluded that juice concentrates from cactus pear could be a suitable coloring food.

Castellar et al. (2008) tested a different technique to prepare a betalain extract through a fermentation process. Purple cactus pear juice (*Opuntia stricta* Haw.) was fermented to improve betanin concentration and significantly reduce the sugar content. The yeast *Saccharomyces cerevisiae* var. *bayanus* was used with good results for this process. After fermentation and centrifugation, the supernatant was concentrated under a vacuum (30 °C).

Therefore, concentrated liquid betanin was obtained, with low sugar content (5.2 °Brix). The final product contained 9.65 g L<sup>-1</sup> betanin and had a color strength of 10.8. As the authors commented, these values were better than those obtained by ethanol/water extraction (Castellar et al., 2006). In addition, bioethanol could be obtained as a by-product. Consequently, and following the current trend of the circular economy, this and other processes involving the cactus pear should be addressed, because of the social and economic benefits they will bring.

Navarro (2018) evaluated, as coloring food, a concentrated juice from the *Opuntia robusta* fruit due to its high betalain content. *Opuntia robusta* is quite a different variety of *Opuntia* fruit, given it is dark purple and round with a thin peel (Fig. 50.1c). The concentrate or coloring food was obtained by vacuum evaporation at a temperature of 30 °C to reach 64–65 °Brix. The betanin concentration of this product was 165 mg 100 g<sup>-1</sup>. The author compared *Opuntia robusta* concentrated colorant with a commercial red beet concentrate, which had a slightly higher value, 185 mg 100 g<sup>-1</sup> of betanin, coming from the root and not a fruit, with the disadvantages that this entails, as indicated above. The cactus pear concentrate showed a total polyphenol content of 3560 mg EAG L<sup>-1</sup> and an antioxidant capacity of 4396 µmol Trolox Eq. L<sup>-1</sup>, both functional properties for a coloring food.

Cassano et al. (2007) clarified by ultrafiltration, *Opuntia ficus-indica* cv. Gialla (orange-yellow) juice, to produce a concentrate by osmotic distillation. Osmotic membrane distillation is a non-thermal membrane process with the potential to concentrate different biomolecules, without damage. The final retentate of the osmotic membrane distillation with a high betalain concentration makes it a promising future source for coloring food. Later the same author (Cassano et al., 2010) also used membrane technologies (microfiltration and ultrafiltration) to obtain betalain juice or concentrate from a yellow cactus pear cultivar. Betaxanthins and betacyanins remained in a high proportion in the retentate, while significant losses were observed for betaxanthins and rejections of the MF and UF membranes towards these compounds were 72 and 77%, respectively, with a betaxanthin concentration of 53.6 and 46.9 mg L<sup>-1</sup> and betacyanins of 40.1 and 34.9 mg L<sup>-1</sup>, for micro and ultrafiltration, respectively. Consequently, the retentate can be used as a raw material to extract betalains or directly to formulate foods.

Compared with the traditional separation methods, membrane technologies as a concentration process have a reasonable cost, and it is a non-thermal separation technique, that doesn't cause thermal degradation of pigments (Sáenz et al., 2012). In recent years, Vergara et al. (2015) clarified a purple cactus pear juice by microfiltration to get a product free of dietary fiber and pulp. For this purpose, two 0.2  $\mu\text{m}$  pore size microfiltration membranes (ceramic and polymeric) were tested. The best results were obtained when the ceramic membrane was used. The permeate was clear, free of turbidity, and had a betacyanins content of 97  $\text{mg L}^{-1}$ . However, some pigments remained in the retentate and could be used directly as a source of betalains and dietary fiber to formulate fiber-enriched beverages. Alternatively, they could be re-suspended in water, and the separation process repeated, to increase the pigment yield. In cactus pear juice in addition to either of the betalains, betacyanins, or betaxanthins, other important components such as polyphenols are found, and consequently, betalains and polyphenols contributed to a product with important antioxidant activity. Given this, the juice colorants from cactus pear fruit can be considered as functional ingredients.

Table 50.1 summarizes the studies about cactus pear betalains (betacyanins and betaxanthins) as a potential colorant or coloring food and applications under different forms in several food matrices, which as far as we know, have been carried out to present.

## 3.2 *Cactus Pear Pulp Encapsulation*

### 3.2.1 **Microparticles**

Betalains are affected during processing and storage by several factors such as temperature, pH, water activity, light, oxygen, among others (Herbach et al., 2006; Khan, 2016). The stabilization of pigments is essential to apply them as colorants in food. Microencapsulation technology is widely used in the food industry to protect bioactive compounds from environmental, food, and also to provide for controlled release (Gharsallaoui et al., 2007). Spray drying is the most used encapsulation method because of its relatively low cost and equipment availability (Gharsallaoui et al., 2007); compared to freeze-drying, spray-drying is 30 to 50 times cheaper (Desobry et al., 1997).

Currently, several spray-drying microencapsulation studies have been published, focusing on process and formulation parameters such as inlet air temperature, outlet temperature, type and content of encapsulating agent and active content. At the same time, response variables such as yield, encapsulation efficiency, pigment recovery, as well as microparticle characteristics among others have been studied (Fernández-López et al., 2018; Gandía-Herrero et al., 2010; Otálora et al., 2015, 2018; Robert et al., 2015; Ruiz-Gutiérrez et al., 2014, 2017; Sáenz et al., 2009, 2015; Vergara et al., 2014).



**Table 50.1** Cactus pear betalains (betacyanins and betaxanthins) as potential colorant, stability, and applications in different food matrices

Specie and pulp color	Colorant form	Colorant betalain content (mg/100 g)		Food matrix application	Colorant/matrices stability	Reference
		Betacyanins	Betaxanthins			
<i>Pulp, juice, extracts, concentrates and non-encapsulated powders</i>						
<i>Opuntia boldinghii</i> Red	Pulp	Total betalains 0.59 mg/100 mL		Citric beverages: Orange (15%), Grape (15%), Cactus pear (5%), Citric acid (0, 0.01, 0.1, 0.5%)	<b>Beverages:</b> 21 days, 7 °C Bet ret.: 61–86%, acidity, pH, sugars, soluble solids: p > 0.05 <b>Sensory preferences:</b> Flavor: 0% citric acid, color: 0% citric acid, taste: 0.1% citric acid	Álvarez et al. (2003)
		Total betalains 6.7 mg/100 g				
<i>Opuntia ficus-indica</i> Purple	Filtered pulp	2.3 mg/100 g (gummies)		Gummy confections	<b>Sensory evaluation</b> Color intensity: high Hardness: medium Cactus pear aroma: medium Cactus pear flavor: medium Acceptability: 10.2 (0–15)	Sáenz et al. (2019)

Specie and pulp color	Colorant form	Colorant betalain content (mg/100 g)		Food matrix application	Colorant/matrices stability	Reference
		Betacyanins	Betaxanthins			
<i>Opuntia ficus-indica</i> Purple and Orange	Pulp	<b>Purple</b> 11.1 mg/100 g <b>Orange:</b> 2.93 mg/100 g	<b>Purple</b> 0.21 mg/100 g <b>Orange:</b> 8.94 mg/100 g	Toppings (modified starch, citric acid, sugar, cactus pear pulp)	<b>Processing stability:</b> <b>Purple</b> Bet ret.: 57% Carotenoids ret.: 10% Total phenolics ret.: 45% <b>Orange</b> Bet ret.: 70% Carotenoids ret.: 2% Total phenolics ret.: 35%	Morales et al. (2009)
<i>Opuntia ficus-indica</i> Purple	Concentrated juice (61.6 °Brix)	100 mg/100 g		Yogurt: (0.2 and 0.3 g/100 g)	<b>Yogurt:</b> 30 days, Appearance, 0.2%:7.1 0.3%: 7.6 Color, 0.2%: 4.9 0.3%: 5.8	Sáenz et al. (2000)
<i>Opuntia robusta</i> Purple	Concentrated pulp (64.7 °Brix)	165 mg/100 g	118 mg/100 g	Yogurt (0.30 g/100 g)	<b>Yogurt:</b> 6 weeks, 4–6 °C, dark conditions Betanin ret.: 50% Betanin degradation rate constant: $1.1 \times 10^{-1}$ weeks <sup>-1</sup>	Navarro (2018)
<i>Opuntia ficus-indica</i> Purple <i>Opuntia megacantha</i> Orange	Concentrated aqueous-alcoholic extracts			Yogurt and milk cream 0.5–2.0 mg/g betanin 0.7–2.8 mg/g indicaxanthin	<b>Lipid oxidation inhibition:</b> <b>Yogurt</b> Indicaxanthin: 23–42% Betanin: 49–88% <b>Cream</b> Indicaxanthin: 7–13% Betanin: 11–57%	Coria-Cayupán & Nazareno (2015)

(continued)

Table 50.1 (continued)

Specie and pulp color	Colorant form	Colorant betalain content (mg/100 g)		Food matrix application	Colorant/matrices stability	Reference
		Betacyanins	Betaxanthins			
<i>Opuntia ficus-indica</i> Orange	Freeze-dried and concentrated pulp (45.1 °Brix)		<b>Freeze-dried</b> 26.4 mg/100 g <b>Concentrate</b> 25.6 mg/100 g	Soft-drink (0.7 g/100 mL)	<b>Food colorants:</b> 30 days, 23 °C, dark conditions No significant variation of betaxanthin concentration <b>ΔE*</b> : Freeze-dried: 5.6 concentrate: 14.9 <b>Soft-drink:</b> 30 days, 4 °C, dark conditions <b>Betaxanthin degradation rate constant</b> ( $\times 10^{-1}$ days <sup>-1</sup> ) freeze-dried: 2.00 concentrate: 1.57 <b>ΔE*</b> : Freeze-dried: 15.7 concentrate: 10.7	Cammona et al. (2019)
<i>Opuntia stricta</i> Red-purple	Concentrate 60:40-ethanol:water-extract (56 °Brix)	473 mg/100 mL		Comparison with natural red colorants concentrates (beet, carrot, and grape skin) and powders (cochineal, elderberry, hibiscus, cabbage)	<b>Concentrate:</b> 80 days $t_{1/2}$ : 25 °C: 61.8 days 4 °C: 236.6 days Similar characteristics to the natural red concentrates (pH, color strength, viscosity, density) <b>ΔE*</b> vs. colorants: (10.2–42.9) Highest chroma (C*), most purple, highest brilliance, second lighter (after hibiscus)	Castellar et al. (2006)

Specie and pulp color	Colorant form	Colorant betalain content (mg/100 g)		Food matrix application	Colorant/matrices stability	Reference
		Betacyanins	Betaxanthins			
<i>Opuntia</i> spp. Red	High pressure carbon dioxide assisted-water extract	Total betalain content 167–190 mg/100 g		Comparison with natural red beet concentrate colorant	Color strength: 5.0, $\Delta E^*$ : 8.4 Lower L*, Higher a*, b*, C*	Nunes et al. (2015)
<i>Opuntia ficus-indica</i> Red	Peel powder Mucilage powder	Total betalains 122.4 mg/100 g		Yogurt (0–10%)	Increase in yogurt antioxidant capacity and bioactive compounds	Hernández-Carranza et al. (2019)
<i>Opuntia stricta</i> Red-purple	Spray-dried powder with glucose syrup	357 mg/100 g		Soft-drink and yogurt	<b>Powder:</b> 1 month, room temperature Color strength ret.: 98% <b>Yogurt/soft-drink:</b> 1 month, 4 °C $\Delta E^*$ : Yogurt: 4.56 (no sugar), 4.44 (sugar) Soft-drink: ~5.	Obón et al. (2009)
<i>Opuntia</i> spp. Red	Peel and seed dried extract Lipophilic microparticles	<b>Dried extract</b> 332 mg/100 g <b>Microparticles</b> 7.5–64 mg/100 g		Ice-cream 1.75 mg/L	<b>Lipophilic particles:</b> Good pink color homogenization <b>Dried extract:</b> Color separation	do Carmo et al. (2015)
<i>Microparticles and encapsulated colorants</i>						
<i>Opuntia ficus-indica</i> Purple	Pulp, ultrafiltered and nanofiltered microparticles with modified starches	31.2– 36.1 mg/100 g	14.0– 16.0 mg/100 g	Soft-drink Microparticles: 12.5 g/L 99.0–99.6% solubility	<b>Soft-drinks:</b> 30 days, 4–5 °C <b>Degradation rates constants</b> ( $\times 10^{-5} \text{ min}^{-1}$ ) Betacyanins: (1.8–3.5) Betaxanthins: (1.1–2.2)	Sáenz et al. (2015)

(continued)

Table 50.1 (continued)

Specie and pulp color	Colorant form	Colorant betalain content (mg/100 g)		Food matrix application	Colorant/matrices stability	Reference
		Betacyanins	Betaxanthins			
<i>Opuntia ficus-indica</i> Orange	Betaxanthin extract microparticles with maltodextrin	12.6 mg/100 g (extract)	27.5 mg/100 g (extract)	Yogurt (1.5 g/100 g) Soft-drink (5.0 g/100 mL)	<b>Microparticles:</b> 6 months, dark conditions, Betax. Ret.: 20 °C: 60%, 4 °C: 90%, -20 °C: 97% <b>Food models:</b> 28 days, 4 °C, <b>Yogurt:</b> Dark conditions Betax. Ret.: 95%, $\Delta E^*$ : 0.95 <b>Yogurt:</b> Light conditions Betax. Ret.: 40%, $\Delta E^*$ : 10.10 <b>Soft-drink:</b> Dark conditions Betax. Ret.: 90%, $\Delta E^*$ : 2.36 <b>Soft-drink:</b> Light conditions Betax. Ret.: 50%, $\Delta E^*$ : 7.90	Fernández-López et al. (2018)
<i>Opuntia ficus-indica</i> Red	Spray-dried and ionic-gelation microparticles			Spray-dried: Yogurt Ionic-gelation: Jelly gum	<b>Food models:</b> 30 days, 4 °C <b>Yogurt:</b> Red $\Delta E^* < 2$ Orange $\Delta E^* < 3$ <b>Jelly gum:</b> $\Delta E^* < 5$	Orálora et al. (2017)
<i>Opuntia megacantha</i> Orange						
<i>Opuntia oligacantha</i>	Pulp extract double emulsions particles	Emulsions total betalains 3–3.5 mg/100 mL		Yogurt (10, 20, 30%)	<b>Emulsions:</b> 36 days Bet: 1.5–2 mg/100 mL <b>Yogurt:</b> 36 days, 4 °C Bet ret.: 71.4–88.4%	Cenobio-Galindo et al. (2019)

Specie and pulp color	Colorant form	Colorant betalain content (mg/100 g)		Food matrix application	Colorant/matrices stability	Reference
		Betacyanins	Betaxanthins			
<i>Opuntia</i> Red	Freeze-dried betalain extracts Soybean lecithin liposomes	Extracts 4.1 mg/L	0.13 mg/L	Soy-based yogurt-like fermented	<b>Soy yogurt-alternative:</b> 21 days, 4 °C Non-encapsulated: Decrease in a* and b* Encapsulated: Decrease in a*, increase in b*	Dias et al. (2020)
<i>Opuntia ficus-indica</i> Red	Clarified juice microparticles with (1-3) (1-4)-β-D-glucan	150 mg/100 g	61 mg/100 g	Maize grits extruded cereal (0, 2.5, 5.0, 7.5%)	<b>Cereal products:</b> ΔE*: 28-55 Increased color, antioxidant capacity, milk, and water absorption indexes Decreased texture and density Sensory preference: 2.5%	Ruiz-Gutiérrez et al. (2017)
<i>Opuntia ficus-indica</i> Purple	Dried ionic-gelation capsules			Gummy candies (30-70%)	<b>Gummies:</b> 30 days, 4 °C, dark conditions ΔE*: (1.3-3.9)	Ojalora et al. (2019)

*Bet* betalain, *AA* ascorbic acid, *Betax* betaxanthin, *ret* retention,  $\Delta E^*$  total color change,  $\Delta L^*$  lightness change,  $\Delta a^*$  red-green color component change,  $\Delta b^*$  yellow-blue color component change, *C*\* chroma,  $t_{1/2}$  half-time

Purple cactus pear (*Opuntia ficus-indica*) pulp and an ethanolic extract were encapsulated with maltodextrin (MD) and inulin (IN) by spray-drying, applying a statistical design. Inlet air temperature and content of biopolymers (140–160 °C and 6–30% for MD; 120–160 °C and 3–15% for IN) were studied to determine optimal conditions (Sáenz et al., 2009). The optimal inlet air temperature was 140 °C and 120 °C for MD and IN microparticles, respectively, and the optimal polymer/extract ratio was 3:1 and 5:1, respectively. Betacyanin and betaxanthin content in microparticles powder were  $4.5\text{--}6.2 \times 10^{-2}$  and  $2.1\text{--}2.9 \times 10^{-2}$  mg 100 g<sup>-1</sup>, respectively. Betalain recovery reached 100% and 67%, for MD-microparticles with pulp and ethanolic extract, respectively or 100% and 86%, for IN-microparticles with pulp and ethanolic extract, respectively.

Purified indicaxanthin solution from *Opuntia* sp. fruit was encapsulated, using maltodextrin as an encapsulating agent (20% w/v) at an inlet air temperature from 120 to 210 °C. Stability during storage was determined as well (Gandía-Herrero et al., 2010). The higher the inlet air temperature, the lower the encapsulation yield (93 to 90%) and the moisture content (8 to 4%). Finally, the optimal inlet air temperature for spray drying was 140 °C and provided a bright yellow powder.

Red cactus pear clarified juice (*Opuntia ficus-indica*) was encapsulated with soluble fiber (1–3) (1–4) β-D-glucan at 15, 22.5, and 30% (w/v) and dried at an inlet air temperature of 160, 180, or 200 °C (Ruiz-Gutiérrez et al., 2014). The encapsulated powder showed betacyanin retention between 73 and 81%, with decreasing values as inlet air temperature increased. Whereas betaxanthin content was not significantly affected, either by inlet air temperature or soluble fiber added to the juice for encapsulation. In a later study, red cactus pear pulp clarified juice was encapsulated with 22.5% (w/v) soluble fiber (1–3)(1–4) β-D-glucan, to obtain microparticles with a betacyanin content of 150 mg 100 g<sup>-1</sup>, and a betaxanthin content of 61 mg 100 g<sup>-1</sup>. Microparticles were used as a natural colorant for extruded cereal (Ruiz-Gutiérrez et al., 2017).

In another study, red-purple cactus pear pulp and ultra-filtered pulp was encapsulated with modified starch Capsul at temperatures from 133 to 219 °C and (pulp or ultrafiltered pulp /Capsul) a ratio from 0.58:1 to 5.42:1 (Vergara et al., 2014). The encapsulation efficiency and recovery reached values of 99.1–99.3% and 70.9–72.4% for betacyanin, and 98.6–98.7% and 70.9–72.4% for betaxanthin, respectively. The yield ranged from 62.6 to 65.3%. The betaxanthin and betacyanin contents were 36 and 16 mg 100 g<sup>-1</sup>, respectively, in microparticles with pulp and ultra-filtered pulp obtained under optimal conditions (133 °C and 3:1 pulp: Capsul ratio, and 2.5:1 (ultrafiltered pulp): Capsul ratio). Sáenz et al. (2015) reported the encapsulation of cactus pear pulp, ultra-filtered and nano-filtered extracts with Capsul and K-4484 tapioca starch. In these systems, the betacyanin and betaxanthin content ranged from 31.2 to 36.1 mg 100 g<sup>-1</sup>, and from 14.0 to 16.0 mg 100 g<sup>-1</sup>, respectively, without statistical differences among the microparticle systems. Moreover, a high encapsulation efficiency for betacyanin and betaxanthin (99.1–99.5 and 98.3–99.7%, respectively) was found, showing a strong betalain-polymer interaction. Purple cactus pear (*Opuntia ficus-indica*) pulp was encapsulated with soybean protein isolate and a protein isolate-polysaccharide blend (maltodextrin or

inulin) (Robert et al., 2015), where the inlet air temperature (100–140 °C) and pulp-encapsulating agent ratio (1:1–5:1) were studied as independent variables. In these microparticles obtained under optimal conditions the encapsulation efficiency of both betacyanin and betaxanthin ranged from 98 to 100%, and the betacyanin and betaxanthin content were 33–45 and 13–18 mg 100 g<sup>-1</sup>, respectively.

Likewise, purple (*Opuntia ficus-indica*) and orange (*Opuntia megacantha*) cactus pear betalain extracts were encapsulated by spray-drying to obtain food colorant, using maltodextrin and cactus cladode mucilage (Otálora et al., 2015; Otálora et al., 2018). Betalain extract was mixed with maltodextrin in a 1:1 w/w ratio, and with maltodextrin-cladode mucilage in a 1:1:0.225 w/w/w ratio. The betanin and betaxanthin content in microparticles reached 49.7–49.5 mg 100 g<sup>-1</sup>, and 34 mg 100 g<sup>-1</sup>, respectively. Recently, a cactus pear (*Opuntia ficus-indica*) betaxanthin extract containing 27.5 mg 100 g<sup>-1</sup> of betaxanthins and 12.6 mg 100 g<sup>-1</sup> of betacyanins was encapsulated with maltodextrin (1:1), with an encapsulation efficiency of 71% and found to be a good source of natural yellow food colorant (Fernández-López et al., 2018).

### 3.2.2 Ionic Gelation

Some other less-common encapsulation techniques, such as ionic gelation have been proposed to prepare food colorants from cactus pear. Purple pulp *Opuntia ficus-indica* betalain extract was encapsulated by external ionic gelation through either calcium alginate (CA) matrix or calcium alginate-bovine serum albumin (CAB) matrix (Otálora et al., 2016). The serum albumin inclusion was intended to assess the effect of the interactions between proteins and carbohydrates on the stability of betalains. The moisture content after air-drying (30 ± 1 °C) of CA and CAB beads was 2.862 ± 0.004% and 2.466 ± 0.002%. The authors concluded that ionic gelation, as an encapsulation method, is more protective than the freeze-drying process, but the serum albumin did not affect the pigment stability. Meanwhile, an orange pulp (*Opuntia megacantha*) betalain extract was encapsulated through the calcium alginate matrix mentioned above, drying the beads at 30 °C for 24 h in a forced-air circulating oven and produced a betaxanthin content of the encapsulated beads of 32 mg 100 g<sup>-1</sup> (Otálora et al., 2018).

### 3.3 Other Cactus Pear Powders

Freeze-drying is another technique used to obtain cactus pear colorants. An example of this was orange cactus pear (*Opuntia ficus-indica*) microfiltered juice that was mixed with maltodextrin in a 5:1 ratio. It was then freeze-dried to obtain a powder containing 4.1 mg L<sup>-1</sup> of betacyanin content and 34.5 mg L<sup>-1</sup> of betaxanthin content that resulted in 93% pigment retention after the drying process (Moßhammer et al., 2006b). Meanwhile, orange cactus pear pulp (*Opuntia ficus-indica*) with



maltodextrin in a 1:1 ratio (pulp-soluble solids) was reported to have a betaxanthin content of 26.3 mg 100 g<sup>-1</sup> after freeze-drying (Carmona et al., 2019).

Obón et al. (2009) studied several variables to obtain an *Opuntia stricta* coloring powder rich in betacyanins through spray drying. Among the variables studied were the drying conditions, the amount of carrier (Glucose syrup (DE 29) needed, the liquid feed rate, and the drying-air inlet temperature. During these trials, not all the glucose syrup/juice ratios gave good results. The stickiness of the powder was a problem, and a minimum amount of carrier (ratio glucose syrup/juice higher than one) was needed to prevent the powder from getting sticky. Thus, optimum conditions for spray drying were: juice content (20% v/v; 1.2 °Brix), glucose syrup content (10% w/v), liquid feed rate (0.72 L h<sup>-1</sup>), spray air flow-rate (0.47 m<sup>3</sup> h<sup>-1</sup>), drying air flow-rate (36 m<sup>3</sup> h<sup>-1</sup>), and inlet drying air temperature 160 °C. The drying yield was (58%), and the powder obtained was high in betanin (357 mg 100 g<sup>-1</sup>) and color strength (4.0).

Bourhia et al. (2020) studied the physicochemical and biochemical characteristics of cactus pear peel powder obtained from Moroccan orange peel varieties, Aakria (*Opuntia ficus-indica*), Derbana (*Opuntia megacantha*), and Mles (*Opuntia ficus-indica*) to explore its potential as a dye for foods. The powder studied at two humidity levels (15 and 10%) showed that  $a_w$  ranged from 0.173 ± 0.002 to 0.336 ± 0.002, being lower, as was expected, than those values from 10% humidity. However,  $a_w$  values can ensure microbial and chemical stability. The powder also showed interesting pigment content (betanin, indicaxanthin, and carotenoids). In some small rural cactus pear seed oil manufacturing plants the peel from the cactus pear fruit goes to waste; so, the valorization of this by-product is highly important to contribute to the circular economy with a new natural ingredient.

## 4 Color and Betalain Stability in Some Cactus Pear Colorants During Storage

### 4.1 Stability of Juice and Concentrate Coloring Foods

Betalain stability, as was mentioned before, is influenced by several factors, such as metals, pH, water activity, light, oxygen, enzymes, and temperature, the latter being the most important degradation factor (Herbach et al., 2006; Stintzing & Carle, 2007).

Castellar et al. (2006) studied the stability (4 and 25 °C) of *Opuntia stricta* concentrate juice (56 °Brix) containing 4.7 g L<sup>-1</sup> betanin. The pigment degradation followed first-order kinetics, showing a half-life time of 61.8 and 236.6 days, at 25 and 4 °C, respectively. The concentration of the extract increased the stability compared with a non-concentrated extract from *O. stricta*, which showed half-life times of 9.9 and 58.4 days at 25 and 4 °C, respectively, under the same storage conditions. The concentration of betalains plays an important role in their stability, so a higher initial content would increase the stability of the pigments over time (Khan, 2016;

Martins et al., 2017) because the degradation of betalains follows first-order kinetics, where the rate depends on the initial concentration of the pigment (Fernández-López et al., 2012).

Obón et al. (2009) reported that the color strength (4.0) and betanin stability of *Opuntia stricta* powder remained stable (98%) during storage at room temperature for 30 days. Carmona et al. (2019) studied different coloring foods from yellow-orange cactus pear pulp (*Opuntia ficus-indica*), either obtained by vacuum concentration (VC) up to  $45 \pm 1$  °Brix and by freeze-drying (FD) using maltodextrin as a carrier in a 1:1 ratio (pulp soluble solids/maltodextrin). Betaxanthin content was  $256.5 \pm 4.8$  and  $263.7 \pm 5.2$  mg indicaxanthin equivalents (IE)  $\text{kg}^{-1}$  in VC and FD, respectively. During storage ( $23 \pm 2$  °C for 30 days), the betaxanthin decrease in VC was not significant, but a perceptible color change ( $\Delta E = 15.0$ ) was detected. However, betaxanthin content and color parameters showed no significant changes in FD colorant, with a color change ( $\Delta E = 5.6$ ) almost imperceptible to the human eye. The stability during the period and at the temperature tested was considered good, but a longer storage period could be worthwhile to study.

## 4.2 Stability of Encapsulated Cactus Pear Colorant

Gandía-Herrero et al. (2010) encapsulated betaxanthins from *Opuntia* sp. fruit, using maltodextrin as an encapsulating agent and then spray dried the sample. The drying temperature was the independent variable to optimize, to increase the performance of encapsulated betaxanthins and their subsequent stability in storage. The authors found betaxanthins encapsulation efficiency to be between 90 and 93%, and pigment retention was 90% in the microparticles stored at  $-20$ , 4, and 20 °C for 6 months in the absence of light.

Fernández-López et al. (2018) stored microparticles from yellow *Opuntia ficus-indica* betaxanthin-rich extract, which exhibited pigment retentions of 60, 90, and 97% at 20, 4, and  $-20$  °C, respectively; meanwhile, *Opuntia megacantha* orange betaxanthin microparticles with maltodextrin-mucilage (1:0.225), showed 60 and 75% retention of the pigments stored at 18 °C and 90 and 57% relative humidity, respectively (Otálora et al., 2018).

On the other hand, purple peel extract from *Opuntia ficus-indica* microparticles spray-dried with maltodextrin and Arabic gum and stored at  $22$ – $25$  °C for 90 days, showed 91–100% betacyanin and 94–100% betaxanthin retention when the former encapsulating agent was used. Meanwhile, when the latter was used, betacyanin retention was 88–100%, and that of betaxanthin was 94–100%, which demonstrates that maltodextrin allowed for greater protection of the encapsulated pigment during storage, under the conditions studied (Toledo-Madrid et al., 2019).

Based on these results, maltodextrin as an encapsulating agent along with low temperature and relative humidity seems to ensure betalain stability during short and medium-term storage periods, which would be promising for the use of these pigments as colorants in shelf-life studies of powdered food formulations. In the

meantime, cactus pear betalain concentrates are more suitable for refrigerated or frozen products since temperatures close to the freezing point are needed to reduce pigment degradation in this form.

Cenobio-Galindo et al. (2019) prepared multiple emulsions (ME) (w/o/w) from cactus pear (*Opuntia oligacantha* C.F. Först) extract encapsulated with two polymers, Arabic gum, and maltodextrin. The total betalain content of emulsions was between 3–3.5 mg 100 mL<sup>-1</sup>, depending on the polymer used, and showed a betalain content reduction to 1.5–2 mg 100 mL<sup>-1</sup> during 36 days of storage. Robert et al. (2020) studied the retention of cactus pear (CP) betanin encapsulated in double emulsions (DE) gelled with gelatin (DE-CP-G) and with gelatin and transglutaminase (DE-CP-GT), as well as in a DE with a liquid external water phase (DE-CP). The betanin degradation rate constant was significantly higher in DE-CP-GT ( $90.2 \times 10^{-3} \text{ days}^{-1}$ ) than in DE-CP-G ( $11.0 \times 10^{-3} \text{ days}^{-1}$ ) and DE-CP ( $14.6 \times 10^{-3} \text{ days}^{-1}$ ) during cold storage (4 °C). A color change to yellow was found in all systems during cold storage (4 °C) and after heat treatment (70 °C/30 min), it was higher in those with GT, which denotes further degradation of betanin. Betalamic acid (bright yellow), *cyclo*-Dopa 5-*O*- $\beta$ -glucoside (colorless), 17-decarboxy-betanin (orange-red), and neobetainin (orange-red) were identified as betanin degradation products. However, in those kinds of double emulsions gelatin protects betanin, making it a potential fat replacer in refrigerated food products.

## 5 Color and Betalain Stability of Cactus Pear Colorants in Food Products

It is well known that appearance is one of the most important attributes for consumer acceptance, and the color stability of a product marks the difference between one colorant and another. In this case, color stability is directly related to betalain (betacyanins or betaxanthins) stability, this being one of the main factors to be considered for its future uses.

The application of betalains as a colorant in food was mainly carried out with soft-drinks and dairy products, such as yogurt. Cactus pear betalains were applied in food as an extract, concentrate, powder, microparticles, among others.

Navarro (2018) colored natural yogurt with a concentrate of *Opuntia robusta* fruit pulp (64.7 °Brix). The yogurt stored (4–6 °C) in the dark for 6 weeks showed betanin retention of 50% and degradation rate constant of  $1.1 \times 10^{-1} \text{ weeks}^{-1}$  that was not significantly different from yogurt colored with commercial beetroot concentrate.

Obón et al. (2009) studied the application in food models (yogurt and soft-drink) of a spray-dried colorant (powders using carrier) from *Opuntia stricta* achieving a purplish-red color, with a small change to red after one month under refrigeration (4 °C) in both food models, with the stability being slightly lower in soft-drinks and suggesting that the pH (3.5) affects the betalain stability.

Fernández-López et al. (2018) used microparticles, spray-dried with indicaxanthin-rich extracts of *O. ficus-indica* fruit pulp and maltodextrin as encapsulating agent, as yogurt and soft drink colorants. The authors reported improved pigment and color stability in beverages and yogurt, with 90 and 95% indicaxanthin retention after 28 days of refrigerated storage in the dark, respectively. Foods exposed to light showed retention of 60 and 50% in yogurt and beverages, respectively. Total color differences ( $\Delta E^*$ ) under dark conditions were 0.95 and 2.36 in yogurt and soft-drinks, respectively, which are slight or not perceptible (Obón et al., 2009); meanwhile, under light exposure, color differences increased up to 10.1 and 7.9, since light induces chromatic changes as a consequence of pigment degradation (Herbach et al., 2006).

Sáenz et al. (2015) evaluated betalain stability in soft-drinks prepared with microparticles from purple cactus pear pulp, ultrafiltered and nanofiltered extracts. The soft-drinks were stored (30 days, 4–5 °C) and kept in the dark. The degradation rate in the soft-drinks, for betacyanins as for betaxanthins, followed first-order kinetics, showing rate constants between  $(1.8\text{--}3.5) \times 10^{-5} \text{ min}^{-1}$  and  $(1.1\text{--}2.2) \times 10^{-2} \text{ min}^{-1}$ , respectively. Furthermore, this formulation also showed a lower color change index during the storage. The betalains from microparticles prepared with cactus pear pulp showed higher stability than those from ultra or nanofiltered extracts.

Betaxanthin from yellow-orange cactus pear vacuum concentrated (VC) and freeze-dried (FD) colorants were applied in soft-drinks models (Carmona et al., 2019). The betaxanthin stability was evaluated during storage at 4 °C. Total color differences ( $\Delta E^*$ ) of 10.7 (soft drink with VC) and 15.7 (soft drink with FD) were recorded at the end of the storage period. The results suggested that FD could be used for coloring instant food (soup or soft-drink powders). VC shows potential for coloring food stored at low temperatures, e.g., chilled matrices such as yogurt or ice creams. Aqueous matrices and low pH ( $\leq 3$ ) are a real challenge for those kinds of natural colorants.

Betalains from *Opuntia megacantha* fruit (purple pulp) encapsulated by spray drying and ionic gelation were applied as a dye in yogurt and jellybeans. The color variation ( $\Delta E^*$ ) was less than 5 at the end of 30 days of storage at 4 °C, which shows the stability of the pigments in food (Otálora et al., 2017, 2019).

The color stability of a betalain-rich extract from red cactus pear in a yogurt-like fermented soy food system stored for 21 days at 4 °C was evaluated by Dias et al. (2020). Extracts were applied directly after freeze-drying and encapsulation in nano-systems (liposomes) with soybean lecithin. In a yogurt-like beverage with non-encapsulated *Opuntia* extract, a decrease in color parameters  $a^*$  and  $b^*$  occurred; while, with an encapsulated extract, the decrease was in  $a^*$  and the increase in  $b^*$ . The results showed that encapsulated *Opuntia* extracts could be an alternative to supplement soy-fermented beverages with betalains, without affecting color and providing health benefits.

Cactus pear (*Opuntia oligacantha* C.F. Först) pulp extract encapsulated in double emulsions (ME) (w/o/w) was added to yogurt, to increase bioactive compounds. Yogurt formulations with different amounts of ME (0%, 10%, 20%, and 30% w/w) were stored at 4 °C for 36 days registering betalain retention between 71 and 88%

and a variation in color parameters from  $-6.6$  to  $-10.3$  for  $L^*$ ,  $-0.1$  to  $-0.4$  for  $a^*$ , and from  $2.0$  to  $0.95$  for  $b^*$ . The total phenolic, flavonoid, and antioxidant activity in the yogurts were protected in gastric and intestinal digestion by the multiple emulsions; so, this yogurt could help to improve consumers' health (Cenobio-Galindo et al., 2019).

Hernández-Carranza et al. (2019) evaluated the addition of dried peel (0–10%) from red cactus pear (*Opuntia ficus-indica* L.) and its mucilage on the color, bioactive compound content, and antioxidant capacity (AC) of yogurt. Results showed that dried cactus pear peel and its mucilage gave a magenta color to the yogurts and increased the bioactive compounds and AC. Yogurt containing 5.5% cactus pear peel and 7.5% mucilage exhibited the highest antioxidant capacity and level of bioactive compounds.

Ruiz-Gutiérrez et al. (2017) applied microparticles of clarified red cactus pear juice as a dye in extruded cereals. The powder was mixed (2.5, 5.0, and 7.5% w/w) with maize grits and extruded. The authors studied the changes in the physicochemical characteristics of the products, concluding that all showed a change in color parameters and the one extruded with the lowest proportion added was like the control without pigment. According to these results, it could be thought that even as an additive, some pigment concentrations tested were high to color those extruded products, i.e. some flavoring could be added to distinguish the flavor since the colors changed from yellow to pink according to the pigment concentration added. Therefore, it would be useful to continue with sensory quality studies on similar extruded products.

Other products that have been developed recently, using cactus pear pulp as a colorant, are gummy confections, characterized by a firm structure with softness and chewiness due to gelatin, starch, or pectin-based gels. Sáenz et al. (2019) prepared gummy confections with filtered purple cactus pear pulp (*O. ficus-indica*), unflavored gelatin, sugar, and water. The antioxidant activity of these types of products included in the enrichment of compounds would be a successful way to deliver health benefits. Two important properties of this type of product are, in addition to its color, its texture, and  $a_w$  that ensures its conservation.

Another application of betalains as an antioxidant was studied by Coria-Cayupán and Nazareno (2015). They applied betanin extract from purple cactus pear pulp (*Opuntia ficus-indica*) and indicaxanthin extract from yellow-orange cactus pear pulp (*Opuntia megacantha*) in yogurt and cream to evaluate the protective effect of cactus betalains against lipid oxidation. The highest protection level of oxidation inhibition was reached using  $2 \text{ mg g}^{-1}$  of betanin and  $2.8 \text{ mg g}^{-1}$  of indicaxanthin for yogurt and cream. These results showed that betanin is more efficient than indicaxanthin for protecting yogurt and cream from oxidation damage and both could be used as a colorant. Color stability was not included in this study.

Sensory evaluation in food products with cactus pear colorant is scarce. The focus of some of these studies is related to product acceptability rather than to the description or quantification of the related-sensorial characteristics (Álvarez et al., 2003; Moreno & Betancourt, 2007).

The sensory evaluation of yogurt with 0.2 and 0.3 g 100 g<sup>-1</sup> concentrated juice from purple cactus pear (*Opuntia ficus-indica*) showed a better acceptance, appearance, and color compared to a control (commercial raspberry flavored yogurt). The sweetness, acidity, aroma, and flavor were not affected by the colorant (Sáenz et al., 2000).

Extruded cereals colored with red cactus pear (*Opuntia ficus-indica*) microparticles were sensory evaluated for texture, color, taste, odor, and preference (Ruiz-Gutiérrez et al., 2017). Texture in cereals with 0 and 2.5% microparticles had the lowest level of hardness and it was classified as “like moderately”. The cereal with 5.0 and 7.5% of microparticles reached the most preferred color but with an increased level of hardness; while, concerning the flavor, consumers preferred the cereal without added microparticles and with 2.5% encapsulated powder, although no smell, in particular, was perceived. Cereal with 2.5% microparticles added was preferred by the consumers when tested alone and with milk because its characteristics were like the control (without pigment). However, it exhibited a better color than the control, demonstrating how the encapsulated powder affected the sensory characteristics of the extruded products.

Recently, Sáenz et al. (2019) reported the results of a sensory quality descriptor evaluation for gummy confections (GC) with purple cactus pear. The trained panel described the GC as having high-intensity color, medium hardness, medium cactus pear aroma, and medium cactus pear flavor, with acceptability considered high for a new product.

Future research should further address the sensory evaluation of foods containing cactus pear colorants or coloring foods, and their effects on aroma, color, flavor, as well as on their acceptance, allowing for the replacement of artificial colorants with these from a natural source.

The cactus pear can be considered as a fruit, or better, a plant that has many possibilities to be fully exploited. Diverse products can be obtained from both the fruit and the pads (Moßhammer et al., 2006a; Sáenz, 2000). The circular economy promotes the recovery of residues or waste and this plant is suitable also for this purpose, as has been explained in this chapter, which highlights the valuable ingredients such as colorants and coloring foods.

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