German Ayala Valencia *Editor*

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I would like to dedicate this book:
To my mother and father ***Amparo*** *and* ***Isauro****, respectively,*
for their example, formation, and immense love
given to me.
To my brothers ***Isauro*** *and* ***Alejandro****,*
for their friendship and love.
To my wife ***Talita*** *for being my twin soul,*
I love you so much!
To my daughter ***Liz****, my little* ***Liz****,*
You are the best of me!
To ***Brazil****,*
for welcoming me and giving me my academic and
professional training.
Germán Ayala Valencia, Ph.D.
Editor

Preface

Food additives are substances used to improve the nutritional value, flavor, and texture of foods, or to preserve them. These substances can be classified as synthetic and natural food additives. Nowadays, consumers are interested in purchasing natural products and processed foods having shorter ingredient lists, familiar ingredients, or minimally processed ingredients. In this context, natural food additives have been broadly studied in recent years as alternatives to synthetic food additives. This book aimed to review the concepts related to natural food additives. In this way, Chapter 1 was focused on defining and classifying natural additives. Chapters 2, 3, 4, and 5 reviewed the use of natural additives such as antioxidants, antimicrobials, colorants, and sweeteners. The potential application of vegetal and microbial sources of natural food additives was studied in Chaps. 6 and 7. Chapter 8 revised the main preservation approaches to stabilize natural food additives. The effect of thermal and nonthermal treatments on the physical, chemical, and biological properties of natural food additives was revised in Chaps. 9 and 10. Chapter 11 studied the toxicological aspects of natural food additives. Finally, Chaps. 12 and 13 were addressed to review consumer attitudes and regulations of natural food additives.

My sincere gratitude to the contributors for their insights into *Natural Additives in Foods*.

Florianópolis, SC, Brazil — Germán Ayala Valencia

Contents

About the Editor

Germán Ayala Valencia received his PhD in food engineering in 2017 from the University of São Paulo, Brazil. He is a professor at the Department of Chemical and Food Engineering in the Federal University of Santa Catarina, Florianópolis, Brazil. He was the guest editor of a special issue published by *Starch – Stärke* (11–12/2021). He is an Early Career Researcher Board Member of the *Journal of Packaging Technology and Science*. He mainly works in the food science and technology area with an emphasis on packaging, pigments, nanotechnology, agro-industrial waste, and encapsulation of bioactive compounds .

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Abbreviations

AA	Ascorbic acid
AAP	American Academy of Pediatrics
ACN	Anthocyanin
ADI	Acceptable daily intake
BBD	Box-Behnken design
B-CN	Beta-casein micelles
BET	Brunauer, Emmett and Teller
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BSA	Serum albumin
BW	Body weight
CBEs	Cocoa butter equivalentes
CCM	Curcumin
CEO	Cinnamon essential oil
CFR	Code of Federal Regulation
CP	Cold plasma
DHAA	Dehydroascorbic acid
DHP	Dynamic high-pressure
DSB	Double-stranded break
DTAB	Dodecyltrimethylammonium bromide
EAE	Enzyme-assisted extraction
EF	Encapsulation efficiency
EFSA	European Food Safety Authority
EOs	Essential oils
EPS	Exopolysaccharides
EU	European Union
FADB	Food Additives Database
FAO	Food and Agriculture Organization
FAS	Fatty acid synthase
FAs	Food additives
FDA	Food and Drug Administration

FD&C	Food, drug, and cosmetic
FDMMs	Mushroom microparticles
FRAP	Ferric reducing antioxidant power
FSSAI	Food Safety and Standards Authority of India
GL	Glycyrrhizin
GE	Genetically engineered
GM	Genetically modified
GMO	Genetically modified organism
GMP	Disodium guanylate
GRAS	Generally recognized as safe
HAS	Serum albumin
HPP	High-pressure processing
HTST	High-temperature, short-time
HVACP	High-voltage atmospheric cold plasma
IMP	Disodium inosinate
IUPAC	International Union of Pure and Applied Chemistry
ISRS	International Society of Rare Sugars
JECFA	Joint Expert Committee on Food Additives
LAB	Lactic acid bacteria
LPO	Lactoperoxidase
LOAEL	Lowest observed adverse effect level
MAE	Microwave-assisted extraction
MAG	Monoammonium glutamate
MEF	Moderate electric fields
MHLW	Ministry of Health, Labor and Welfare
MSG	Monosodium glutamate
NOAEL	No observed adverse effect levels
NFAs	Natural food additives
ORAC	Oxygen radical absorption capacity
OS-GTE	Oil-soluble green tea extract
PA	Phytic acid
PEF	Pulsed electric field
POD	Peroxidase
PL	Pulsed light
PLA	Polylactide
PPO	Polyphenol oxidase
PUFA	Polyunsaturated fatty acids
QCT	Quercetin
RONs	Reactive oxygen and nitrogen species
RSM	Response surface methodology
SCFA	Short-chain fatty acids
SFE	Supercritical fluid extraction
SGD	Saccharomyces Genome Database
SGF	Simulated gastric fluid
SGs	Steviol glycosides

SFAs	Synthetic food additives
TA	Tannic acid
TAG	Triacylglycerols
TAL	Tyrosine ammonia lyase
TBARS	Thiobarbituric acid
TBHQ	Tert-butylhydroquinone
TE	Trapping efficiency
TFC	Total flavonoids content
TPC	Total phenols content
TPP	Tripolyphosphate
TRAP	Total free radical capture antioxidant parameters
UAE	Ultrasound-assisted extraction
Us	Ultrasound
US	United States
WHO	World Health Organization
WPI	Whey protein isolated
YE	Yeast extract

Chapter 1
Food Additives: Importance, Classification, and Adverse Reactions in Humans

Jennyfer Flórez-Méndez and Jessica López

1.1 Introduction

Changes in the lifestyles of families, together with the gradual abandonment of the habit of buying food almost daily and preparing food immediately before meals, have occurred due to the rapid development of food technologies, including the use and increasing development of food additives (FAs). FAs have become indispensable in the production of processed foods and they are increasingly relevant in providing the "convenience" that consumers seek [1]. This trend has led to a rapid increase in the production of numerous FAs, both of synthetic and natural origin [2]. In addition, FAs are essential for the food industry to provide food that reaches the market in compliance with increasingly demanding legal and consumer requirements [3].

FAs refer to all kinds of trace substances used in food or food processing, the amount of which does not exceed 2% of the total weight of the food. The use of these substances continues to be the most unknown in the food industry [4]. FAs are in practically all the foods and beverages we eat, and they are part of our daily lives. Although FAs have been associated with modern times, they have been used for centuries. The Codex Alimentarius defines a food additive as follows: "*Any substance which as such is not normally consumed as a food, nor is it used as a basic ingredient in food, whether or not it has nutritive value, and which is as a basic ingredient in food, whether or not it has nutritive value, and the intentional addition of which to food for technological (including organoleptic) purposes at the stages of manufacture, processing, preparation, treatment, packaging, wrapping, packing,*

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transport or storage, results or may result in the or storage, results or may reasonably be expected to result (directly or indirectly) in the (directly or indirectly) by itself or by-products thereof, into a component of the food or an element affecting its characteristics". This definition does not include "contaminants" or substances added to food to maintain or improve nutritional qualities [5]. The food industry employs about 25 classes of FAs, which are used according to the specific legislation of each country and following a food safety policy based on the Codex Alimentarius [6].

The word "additive" generally refers to a preservative. Preservation of foods using additives is an important method for protecting foods against deterioration caused by microbial activity and oxidation, thereby extending the shelf life and ensuring their safety. However, FAs are viewed negatively by consumers, and foods containing FAs are regarded as being unsafe or of low quality [7].

The use of FAs in food processing has been increasing, and currently, some 25,000 different FAs are used worldwide [8], each one with a different purpose in the food matrix: preservatives, antioxidants, carriers, acidifiers, acid regulators, anticaking agents, antifoaming agents, bulking agents, emulsifiers, emulsifying salts, firming agents, flavor enhancers, foaming agents, gelling agents, glazing agents, humectants, modified starches, packaging gases, propellants, raising agents, sequestrants, stabilizers, thickeners, flour treatments agents, and contrast enhancers [9]. These substances can be obtained from biological sources such as plants, animals, and minerals or produced synthetically. The most common additives used in foods are aspartame, frequently used in beverages as a sweetener or preservative [10]; sodium nitrite, broadly utilized in sausages as preservative and flavor enhancer [11]; caramel, regularly used as a sweetener or as a food colorant; monosodium glutamate (MSG) employed in Asian food as a flavor enhancer and preservative [12]; glucose, the most popular additive used in the food industry as a sweetener; vitamin E, used in the food industry as a vitamin, and beta carotene utilized as a food colorant [13]. FAs are essential for the food industry to make food that meets the increasingly challenging market and legal demands [7]. Bioactive additives, such as antioxidants, antimicrobials, flavors, and probiotics, represent an upcoming technological challenge for the food industry. This chapter aims to analyze the state of the art regarding the use of NFAs and SFAs and their food applications, focusing especially on their impact on human health.

1.2 Importance of Using Additives in Foods

FAs cover a wide range of substances, from common ones such as sodium bicarbonate used to make cakes in the domestic kitchen, to mono- and di-acetyltartaric esters of mono- and diglycerides of fatty acids used as emulsifiers in commercial bread production. They include curcumin, the yellow color in turmeric, beeswax and citric acid, the acid in citrus fruit, as well as substances prepared synthetically. It has long been fashionable in the media to criticize additives and, in so doing, to lump them all

together. However, this ignores their diversity, their vital role in food production and preservation, and the extensive testing they have undergone before being approved [14].

FAs play an important role in the modern food processing and supply system, enabling a year-round supply of healthy and palatable food products for a growing urban population [8]. Consumers want, among other things, good quality at a low price, easy preservation and culinary preparation of foods, and minimal change in products over time, without loss of flavor, color, and tenderness. To achieve this goal, food companies are forced to use FAs within the legal framework [15].

The presence of FAs is found in all kinds of foods, from the minimally processed to the highly processed and transformed foodstuff. The interaction between some FAs and people has not been without controversy. In the '80s, FAs were considered dangerous to be consumed, which fueled generalized fear and led to the removal of some additives, namely colorants from processed foods [3].

A look at the shelves of any supermarket in the developed world will reveal a vast range of foods, of different flavors, colors, and textures from many cuisines, which pay tribute to the skill of chefs, scientists, and engineers in the food industry and the companies that provide them with ingredients and additives. What we now call FAs are the result of over 2000 years of creativity in the food sector. Once the man had progressed from nomadic hunter-gatherers to living in settled communities he needed to work out how to store food for times of scarcity [14]. Ancient peoples used salt for preserving meat and fish, enhanced the flavor of some foods by adding spices and herbs, and preserved fruits using sugar, and cucumbers by pickling them in vinegar. Nowadays, consumers demand food products that are nutritious, flavorful, convenient, safe, colorful, and affordable [8].

The use of FAs is only justified if it responds to a technological need, does not mislead the consumer, and is used for a well-defined technological function, such as preserving the nutritional quality of foods or improving their stability. Additives in food products can provide many specific characteristics. Although intentionally added to foods, they could be related to the onset of diseases. Additives may also undergo unintended interactions with other additives and food constituents, with desirable or undesirable consequences on food quality and human health. In this sense, the health benefits of artificial sweeteners have been questioned in recent years. The World Health Organization (WHO), in cooperation with the Food and Agriculture Organization of the United Nations (FAO), studied the risks to human health of FAs [5]. The body responsible for this evaluation is the Joint FAO/WHO Expert Committee on FAs (JECFA), an international and independent group of scientific experts. Currently, the use of FAs is the subject of controversy related to the potential impacts of these compounds on human health, with several studies reporting on the risks of consuming additives [16–21]. In this line, numerous FAs have been studied in the last years, but sweeteners have led to the greatest controversy since the increase in their consumption has been on the rise in recent years due to fitness trends. Nevertheless, several studies have demonstrated that sweeteners could be correlated with the development of diseases [10, 22–24]. The consumption of artificial sweeteners has been reported to reduce the diversity of the gut microbiome and impair glucose metabolism, resulting in glucose intolerance [25]. The recent

discovery that non-caloric sweeteners also seem to increase the prevalence of diabetes and weight, unravels a grim future for patients with sugar restrictions and the average consumer. Some studies suggest that non-nutritive sweeteners can, surprisingly, be related to weight gain and risk of type 2 diabetes through 3 potential mechanisms: (a) interference with learned responses that contribute to control glucose and energy homeostasis; (b) interference with the gut microbiota, inducing glucose intolerance; and (c) interaction with sweet-taste receptors that may trigger insulin secretion [26, 27]. Public health organizations have made recommendations to limit nutritive sweeteners added to products due to their potential contribution to chronic disease Human diseases risk [28]. In addition, some FAs have been demonstrated to induce dysbiosis, which leads to the development of gut and gastrointestinal diseases [29]. Furthermore, colitis, metabolic dysregulation, obesity, and its related comorbidities have all been found to be associated with the improper use of FAs [30–32]. In the same way, there is growing evidence that some chemicals found in food colorings, preservatives, and packaging materials may harm children's health, according to the new American Academy of Pediatrics (AAP), whose policy statement which calls for an urgent reform of the U.S. food additive regulations [33].

The effects of the different types of additives on human health are, among others, related to the method for elaborating them. Although chemical synthesis tends to be cheaper, potential health risks associated with this process remain a concern. Enzymatic production of these compounds has received much attention as it offers notable advantages over chemical processes. Although several enzymes have been reported to be effective for the biosynthesis of flavors and FAs, industrial production of these compounds using enzymes remains unpopular [34].

The urgent need for a comprehensive understanding of FAs, including their molecular structures, biological activities, and precise toxicological evaluations, prompted the creation of the AdditiveChem database (http://www.rxnfinder.org/additivechem/). This database has curated >9064 types of FAs, along with their molecular structure, chemical, and physical properties, absorption, distribution, metabolism, excretion, and toxicity properties, biosynthesis and biodegradation methods, usage specifications, toxicological and risk assessment data, and targets in the human body from 16 databases to construct an efficient search platform for in silico preliminary evaluations. AdditiveChem database enables an exploration of the relationship between the structure and function of FAs [35], as well as The Food Additives Database (FADB), which offers detailed 3D structures and the chemical and physical properties of 2540 FAs [36].

1.3 Additive Classification

Additives can be classified according to different criteria, but they are often categorized by their technological function. Table 1.1 presents the different types of additives grouped by functional class and subclass, with a brief explanation of their technological function in the food in which they are incorporated.

Table 1.1 Additive classification: Functional class, subclass, and technological functions

Functional class	Subclass	Technological function
Acidity regulators	Acidity regulators	Alter or control the acidity or alkalinity of foods for stability and prevent spoilage.
	Regulating agents	
	Acid base	
	Alkali	
	pH adjusting agents	
Anticaking agents	Anticaking agents	Reduce the tendency of food particles to adhere to each other and prevent moisture absorption.
	Drying agents	
	Antistick agents	
	Dusting agents	
Antifoaming agents	Antifoaming agents	Prevent or reduce foaming in foods.
	Defoaming agents	
Antioxidant agents	Antioxidants	Extend the shelf life of food products by protecting them against deterioration caused by fat oxidation (rancidity) and color changes.
	Antioxidant synergist	
	Sequestrants	
	Anti-browning agents	
Bleaching agents	Bleaching agents	Additives used (not in flours) to discolor foods. Bleaching agents do not contain pigments.
Bulking agents	Bulking agents	Increase the volume of foods without contributing significantly to their available energy value and taste.
	Filler	
Colorings	Decorative pigment	Provide or restore food color.
	Color pigment	
	Surface colorant	
Color retention agents	Color retention agents	Stabilize, retain, or intensify the food color.
	Color fixative	
	Color stabilizer	
	Color adjunct	
Emulsifiers	Emulsifier	Form or maintain a uniform emulsion of two or more phases in foods.
	Plasticizer	
	Dispersing agent	
	Surface active agent	
	Crystallization inhibitor	
	Density adjustment agent	
	Clouding agent	
	Suspension agent	
Emulsifying salt	Emulsifying salt	Rearrange the proteins in foods to prevent their fat separation.
	Melding salt	
Firming agents	Firming agent	Maintain food firmness and crispness.
Flavor enhancers	Flavor enhancer	Enhance the flavor of foods (not providing flavor of their).
	Flavor synergist	

(continued)

Table 1.1 (continued)

Functional class	Subclass	Technological function
Flour treatment agents	Flour treatment agent	Improve the baking quality or color in doughs.
	Flour bleaching agent	
	Flour improver	
	Dough conditioner	
	Dough strengthening agent	
Foaming agents	Whipping agent	Form or maintain a homogeneous dispersion of a gaseous phase in a liquid or solid food.
	Aeration agent	
Food acids	Acidulants	Increase the acidity or impart a sour taste in foods.
Gelling agents	Gelling agent	Improve food texture by forming gels.
Glazing agents	Glazing agent	Provide a protective coating or shiny appearance to foods.
	Sealing agent	
	Coating agent	
	Surface-finish agent	
	Polishing agent	
	Film forming agent	
Humectants	Humectant agent	Protect foods from moisture loss, may be used in the formulation of foods susceptible to dryness.
	Wetting agent	
	Moisture/water retention agent	
Leavening agents	Leavening agents	Promote rising of the baked good, i.e., they release gas and thereby increase the volume of doughs and batters.
Preservatives	Preservative substances	Extend the shelf life of foods by protecting them from spoilage caused by mold, fungi, bacteria, or yeast (antimicrobials).
	Antimicrobial preservatives	
	Antimycotic agent	
	Bacteriophage control agent	
	Fungistatic agent	
	Mold inhibiting agent	
	Antimicrobial synergist	
Propellants	Propellants	Gaseous food additives that expel foods from a container.
Sequestrants	Sequestrants	Substances forming chemical compounds with metal ions, through the complexation of metals ions that catalyze hydrolytic reactions and degradations process in foods.

(continued)

Table 1.1 (continued)

Functional class	Subclass	Technological function
Stabilizers	Binders	Allow the homogenous dispersion of two or more immiscible materials, stabilize the emulsions and suspensions, improve the texture of food, stabilize the color and flavor, and improve the overall quality and acceptability of the food.
	Firming agents	
	Moisture/water retention agent	
	Foam stabilizers	
	Emulsion stabilizers	
	Color stabilizers	
	Colloidal stabilizers	
Sweeteners	Sweeteners	Substances other than sugar and used to improve the sweet taste in foods with or without extra calories.
	Intense sweeteners	
	High-intensity sweeteners	
Thickeners	Thickeners	Increase food viscosity without substantially modifying other properties.
	Bodying agent	
	Binders	
	Texturizing agent	

1.3.1 Acidity Regulators

Acidity regulators are used to alter and control the acidity and alkalinity of foods, thereby influencing sensory perception, processing, and food safety [37]. These compounds are constituted by inorganic acids, organic acids, conjugated salts and bases, and can act in conjunction with the buffering system or alone [6].

The organic acids mainly used as acidity regulators are citric acid, ascorbic acid, benzoic acid, tartaric acid, acetic acid, formic acid, malic acid, and succinic acid. Among the acidity regulators widely used in the food industry are malic acid, lactic acid, phosphoric acid, fumaric acid, and citric acid [38].

1.3.2 Anticaking Agents

The caking of powdered foods occurs due to internal and external factors, for example, hygroscopicity, water content, moisture, and air temperature. Anticaking agents prevent powdered foods from becoming lumpy, permitting a free-flowing condition and making these products manageable for packaging, transport, and for use by consumers [6].

Anticaking agents can be classified according to their origin into natural (such as kaolin, talc, and bentonite/silicate material) and synthetic (when manufactured from raw materials such as silicon dioxide) [39]. Among the anticaking agents typically

used in the food industry are calcium silicate, silicon dioxide, iron ammonium citrate [40]. Calcium silicate is a hydrous or anhydrous inorganic material used as an anticaking agent in the food industry. It is prepared by different reactions between siliceous material and calcium compounds. It can be obtained from naturally occurring limestone and diatomaceous earth or produced synthetically from silicon dioxide and calcium oxide with various ratios. Silicon dioxide is defined as an amorphous substance called fumed silica or hydrated silica according to the production method. Two different manufacturing processes are applied for synthetic amorphous silica production (thermal and wet processes). Iron ammonium citrate is a complex salt with an indeterminate structure consisting of iron, ammonia, and citric acid [41].

Nurhadi et al. [39] indicated that the mechanism of anticaking agents might be because these compounds compete for water with the host material, acting as a water barrier, eliminating surface friction and inhibiting crystal growth.

1.3.3 Antifoaming Agents

Antifoaming agents prevent or reduce foaming by maintaining uniform aeration of gases in foods. These compounds destabilize the liquid film that covers the air bubble, displacing the substances from the surface, thereby preventing foam stability [6].

Three possible mechanisms have been proposed as to how defoaming agents work: (a) that the antifoam agent displaces a surface active compound from the interface, thereby stopping it from stabilizing the foam; (b) that the antifoam acts to form hydrophobic 'bridges' between interfaces, causing the liquid film to rupture and hence collapsing the foam; (c) that droplets of antifoam can spread throughout the liquid film, thinning it and causing it to rupture [6]. An example of antifoaming agents is polypropylene glycol, silicone, and soybean oil.

1.3.4 Antioxidant Agents

The food industry tries to prevent food oxidation by using different techniques, ranging from hermetic vacuum packaging to the use of substances with antioxidant properties. Oxidation is a limiting factor in the quality and acceptability of foods, as well as their main degradation process, limiting shelf life [6, 42]. Antioxidants can be defined as "*a substance that, which at low concentration delays the oxidation of proteins, carbohydrates, lipids, DNA, cell membrane, and other cellular components*" [43]. There are different types of antioxidants, the primary antioxidants are known as radical scavengers; quenchers, which deactivate high-energy oxidant species; oxygen scavengers, that remove oxygen from systems, avoiding their destabilization; chelators, that bind to metals and prevent them from initiating radical formation; and the antioxidant regenerators, that regenerate other antioxidants when these become radicalized [3].

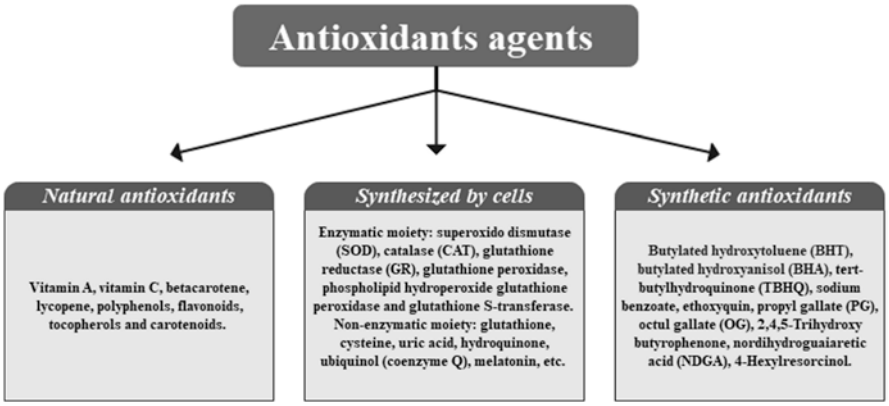

Fig. 1.1 Classification of antioxidants

Antioxidants can be classified as natural antioxidants, antioxidants synthesized by cells, and synthetic antioxidants (Fig. 1.1) [43].

1.3.5 Bleaching Agents

These are peroxides, broadly used to whiten foods such as fruits and cheese [44]. A bleaching agent is a material that lightens or whitens a substrate through chemical reactions. The bleaching reactions usually involve oxidative or reductive processes that degrade color systems [45]. An example of a bleaching agent is hydrogen peroxide, which is a colorless liquid, used mainly as a whitening agent for different foods, including oilseed meals [46].

1.3.6 Bulking Agents

Bulking agents increase the volume of food without contributing significantly to its available energy value. The most common bulking agent is starch, used to increase the bulk of foods without affecting their nutritional value [44]. Mannitol, methylcellulose, microcrystalline cellulose and polydextrose are some common bulking agents introduced into foods such as frozen dairy desserts [6].

1.3.7 Colorings

The US Food and Drug Administration (FDA) defines color additives as *"any dye, pigment, or other substance that can impart color to a food, drug, or to other pharmaceuticals, cosmetics, or the body"* [47]. The purpose of using these additives is

to offset the color loss of the food products due to environmental factors as a result of exposure to moisture, air, light, temperature extremes, and storage conditions. Colorings also enhance colors that occur naturally, correct natural variations in color and provide color to the colorless [40, 48].

Coloring agents are classified according to their origin into natural and synthetic. Table 1.2 summarizes information about the most consumed coloring agents by category.

Table 1.2 List of the most consumed food colorings by category

Food colorant type	Name and code	Additive description	References
Natural	Carminic Acid (E-120)	Natural red colorant obtained from the body of the insect *Dactylopius coccus* costa. Its major allergen is cc38k. In order to obtain this dye, it is necessary to dry and spray the body of pregnant females of these insects. This dye is called by the FDA "cochineal extract" or "carmine" and is classified as exempt from certification. It is used in canned vegetables, jams, meats, dairy products, sausages, alcoholic beverages, soft drinks, and so forth	[49, 50]
Natural	Annatto (E-160b)	Natural colorant obtained from the seed fruit of the tropical shrub *Bixa orellana*. The color ranges from yellow to orange. The carotenoids bixinand and norbixin are the main phytochemical constituents of Annatto. The fat-soluble part of the extract is bixin and the water-soluble part is norbixin. It is used in coffee cream, vanilla ice cream, fish, snacks, meat, rice, cheeses, margarine, butter, rice, smoked products, and so forth	[48, 49]
Natural	Lutein (E-161b) and astaxanthin (E-161j)	Carotenoids used in nutraceuticals products and pharmaceutical applications. These carotenoids offer yellow, orange, and red colors to the products. Plants, aquatic animals, algae, and fungus are the sources of these carotenoids	[48]
Natural	Paprika (E160c)	Natural colorant that constitutes the carotenoids capsanthin and capsorubin. It is also used to impart yellow and orange colors in foods	[48]
Natural	Curcumin (E-100)	Natural pigment of turmeric extracted from the dried rhizomes of *Curcuma longa*. Curcumin is used to impart orange color in mustard, yogurt, baked goods, dairy industry, ice creams, salad dressings, and so forth	[48]
Natural	Riboflavin (E-101)	Part of the vitamin B group. It is a yellow-orange solid substance with poor solubility in water. This food coloring is present in a wide range of foods, with liver, milk, meat, and fish being the most important sources. Riboflavin can be obtained by controlled fermentation using a genetically modified strain of *Bacillus subtilis* or the fungus *Ashbya gossypii*	[48]

(continued)

Table 1.2 (continued)

Food colorant type	Name and code	Additive description	References
Natural	Indigotine (E-132)	It is a glycoside of indoxyl extracted from the leaves of the plants *Indigofera tinctoria*, *Indigofera suifruticosa*, and *Isatis tinctoria*	[50]
Syntethic	Indigo Carmine (E-132)	Indigo Carmine is a blue synthetic colorant commercialized as dark-blue powder or as granules, it is soluble in water and broadly applied in confectionery, teas, batters, ice cream, candies, flavored drinks, cookies, and so forth	[49]
Syntethic	Cochineal red/ Ponceau red (E-124)	Red synthetic colorant substitute of natural cochineal. It is used in confectionery, sausages (salami), jams, dairy products, sweets, and so forth	[49]
Syntethic	Tartrazine (E-102)	Synthetic colorant of lemon yellow in color and it is a type of anionic azo dye. It is soluble in water and used to impart yellow color. In combination with brilliant blue, it produces green color. Tartrazine is mainly used in food products as: ice cream, ice pops, popsicles, confectionery, crackers, mustards, mayonnaise, soft drinks, liquors, alcoholic beverages, cheeses, sausages, pastas, hard candy, and so forth	[48–51]
Syntethic	Erythrosine (E-127)	Synthetic food colorant based on polyiodinated xanthene used to impart cherry-pink color. It is used in candies, ice cream, popsicles, cake-decorating gels, and so forth	[48–50]
Syntethic	Sunset yellow or orange yellow (E-110)	Synthetically coal tar derived from azo dye. The color ranges from yellow to orange. It is mainly used in fermented products, orange squash, orange jelly, marzipan, dairy products, apricot jam, citrus marmalade, lemon curd, sweets, and so forth	[48, 49]
Syntethic	Allura red (E-129)	It is a dark-red, water-soluble, and azo dye used as food dye for the replacement of amaranth. Allura red is originally derived from petroleum. It is added into soft drinks, children's medications, and cotton candy	[48]
Syntethic	Brilliant blue (E-133)	Synthetic and water-soluble dye derived from coal tar. It has a reddish-blue appearance. As a blue dye, it gives green shades when combined with tartrazine. Brilliant blue is used in the preparation of food items as ice cream, tinned processed peas, dairy products, sweets, drinks, and so forth	[48, 50]
Syntethic	Brilliant black (E-151)	It is a synthetic, water-soluble, and diazo dye mainly used for food coatings, desserts, sweets, ice cream, mustard, red fruit jams, soft drinks, flavored milk drinks, fish, paste, and so forth	[48]
Syntethic	Fast green (E-143)	It is a triarylmethane food dye mainly used in tinned green peas, jellies, sauces, fishes, desserts, and dry bakery mixes	[48]

cc38k 38-kD protein, *FDA* Food and Drug Administration

1.3.8 Color Retention Agents

Color retention agents are FAs used to stabilize, retain or intensify food color. In contrast to colorings, color retention agents are used to preserve a food's existing color [52]**.** Some examples of color retention agents are magnesium hydroxide, magnesium hydroxide carbonate; nitrates, and ferrous lactate [6].

1.3.9 Emulsifiers

Emulsifiers are additives used to stabilize food emulsions of two or more phases [53]. An emulsion is a colloidal system that consists of small oil droplets suspended in an aqueous phase, whose dispersion medium and dispersed phase are both liquids. By adsorbing onto droplet surfaces and lowering the interfacial tension, emulsifiers tend to produce an emulsion. Once it gets produced, it turns into a facile breakup of the droplet [54]. If the oil phase disperses in the aqueous phase, the phenomenon is known as an oil-in-water emulsion (O/W), and if the aqueous phase disperses in the oil phase, it is known as a water-in-oil emulsion (W/O) [55].

Emulsifiers have both hydrophilic and hydrophobic moieties and thus reduce interfacial tension between the oil and water phases, preventing suspended droplets within the emulsion from undergoing the processes of separation, flocculation, creaming, sedimentation, or coalescence [53].

Emulsifiers play an essential role in the physicochemical properties of food products, affecting their stability, texture, and shelf life [6, 56]. Their use provides the permanence of the sensorial, physicochemical and rheological properties of the food products by providing a uniform dispersion between the dispersed (droplets) and continuous (bulk) phases, thereby promoting stable heterogeneous systems, such as water and oil mixtures [6].

Emulsifiers can be classified as synthetic, natural, finely dispersed solids, and auxiliary agents based on their chemical structure. Finely dispersed solids increase the viscosity of the dispersed phase and reduce the interaction between the dispersed particles by causing swelling and the formation of a particulate layer around the dispersed phase particles. These compounds are mostly used in the formation of O/W emulsions. Auxiliary agents include various fatty acids (e.g., stearic acid), fatty alcohols (e.g., stearyl or cetyl alcohol), and fatty esters (e.g., glyceryl mono stearate). Since their emulsifying properties are rather poor, these compounds should be combined with coemulsifiers [55].

In turn, synthetic emulsifiers are classified as anionic, cationic, nonionic, and amphoteric. Some examples of synthetic emulsifiers are diacetyl tartaric acid esters of mono glycerides, dodecyltrimethylammonium bromide (DTAB), polysorbate, sorbitan monolaurate (spans 20), and polyoxyethylene sorbitan Monooleate (Tween 80).

In addition, the most commonly used natural emulsifiers are proteins, phospholipids, polysaccharides, lipopolysaccharides, commonly used bioemulsifiers (e.g., saponins, sophorolipids, rhamnolipids, and mannoproteins), and bioemulsifiers isolated from plant materials or produced by fermentation using bacteria, yeasts or fungi [55].

1.3.10 Emulsifying Salt

These additives rearrange the proteins, producing a homogeneous fat distribution, preventing their separation from food. Different emulsifying salts contribute differently to processed food quality, sensory and rheology [57]. Some examples of emulsifying salts are sodium dihydrogen phosphate, phosphates, potassium dihydrogen citrate, sodium aluminum phosphates, sodium lactate, tripotassium citrate, and trisodium citrate.

1.3.11 Firming Agents

Firming agents, which provide firmness, can be used to reinforce the structure of foods by means of stabilizing the cellular structure of fruits and vegetables or interacting with gelling agents to produce or reinforce food gels [6].

Among them, calcium salts such as chloride, citrate, sulfate, lactate, and phosphate have been widely used to enhance the effects of hardening before canning and freezing fruits and vegetables [58].

1.3.12 Flavor Enhancers

Flavor enhancers are a class of FAs used to enhance the sensory characteristics of foods, especially their taste and flavor [59]. They may be extracted from natural sources and are compounds characterized by the presence of the amino acid glutamate, or by nucleotides inosinate and guanylate. Those substances can increase salivation and facilitate the dissolution of food, providing a favorable chemical environment for the perception of taste by the recipient cells [60].

The monoammonium glutamate (MAG), monosodium glutamate (MSG), and nucleotides composed of disodium inosinate (IMP), disodium guanylate (GMP), and others, are the most common flavor enhancers used by the food industries MSG is widely incorporated in foodstuffs to enhance taste and palatability [59, 60].

1.3.13 Flour Treatment Agents

Flour treatment agents are added to flour to improve its color and use in baking [48]. Usually, they both bleach and "mature" the flour, being important in the flour milling and bread-baking industries. Chemical agents used as flour improvers are oxidizing agents, which may participate in bleaching and dough improvement [44]. Some flour treatments agents include benzoyl peroxide (used only for flour bleaching), chlorine gas, chlorine dioxide, nitrosyl chloride, and nitrogen di and tetra oxides (used for bleaching and improving dough) and oxidizing agents used exclusively for dough improvement are potassium bromate, potassium iodate, calcium iodate, and calcium peroxide. Bleaching agents like sulphites and benzoyl peroxides improve the baking of the flour products without changing the color of the foodstuffs [6].

1.3.14 Foaming Agents

Foams are classified as colloidal dispersions where a gaseous phase is dispersed in a continuous aqueous phase. They are found in many foods, for example, ice cream, cakes, mousses, and whipped creams. In most of them, proteins are the main surface-active agents, contributing to their formation and stabilization. Foaming ability is an important characteristic of proteins and other amphoteric molecules [61]. Some examples of foam stabilizers protein are bovine serum albumin (BSA), human serum albumin (HSA), and casein.

Foaming agents are employed in the food industry for the stabilization of foams produced in manufacturing steps, which are a prerequisite in the quality control of various foodstuffs [6]. These compounds provide the texture to many aerated food products. Thus, knowledge of the mechanism of foam formation and stabilization is essential if a foam of the required characteristics is to be produced [62].

1.3.15 Food Acids

Food acids are substances added to foods to increase their acidity or to impart a sour taste. These compounds also act as preservatives and antioxidants. In the food industry, the most common food acids used are citric acid, tartaric acid, malic acid, fumaric acid, and lactic acid [63].

Organic acids are natural antimicrobials used extensively in the food industry to inhibit microbial growth [64]. Fumaric acid is one of the compounds reported to inhibit the growth of bacteria such as *Escherichia coli, Staphylococcus aureus, Salmonella spp*., and *Clostridium botulinum* [65]. For example, this food acid is currently used in wheat and corn tortillas, as well as in sourdough, rye breads,

refrigerated biscuit doughs, fruit juice, nutraceutical drinks, gelatin desserts, gelling aids, pie fillings, and wine. Due to its low molecular weight, fumaric acid has more buffering capacity than other food acids at pH ≈ 3.0 [66].

1.3.16 Gelling Agents

Gelling agents are food additives used to promote gel formation, which is an intermediate state between solid and liquid foods [67]. Some stabilizers and thickening agents are also gelling agents. The gelling agents produce a gel with suitable stability, which can be a quality indicator, and act in the processing steps to enrich the viscosity of the final products [6].

Natural gums, gelatin, carrageenan, proteins, starches, pectin, and agar-agar are the most commonly gelling agents used for food applications [67, 68]. Among them, pectin is the main gelling agent used in jellies and marmalades. Addition of gelling agents is important to ensure that the jam product has a reasonably thick consistency and is firm enough to hold the fruit puree-sugar in position [69].

1.3.17 Glazing Agents

Glazing agents are FAs used to improve the appearance of foods. Furthermore, glazing agents can protect foods since they can be used as food coatings [70]. The most common glazing agents used are beeswax, candelilla wax, carnauba wax, castor oil, and polyethylene glycol, among others. Gum Arabic is also used as a glazing agent in combination with a fine blending of powdered sugar in the manufacture of lozenges [6, 70].

1.3.18 Humectants

Humectants are hygroscopic substances used to keep foods moist due to the presence of one or more hydrophilic groups such as hydroxyl (-OH), amine ($-NH_2$) and carboxyl (-COOH), in their structure [71].

In bakery products, humectants are often used as binding/entrapping agents in order to keep moisture locked into the food during its shelf life. Humectants most commonly used in the food industry are glycerol, mannitol, polydextrose, and propylene glycol [6, 71]. These compounds are sugar alcohols detected in plant products such as berries and fruits, but no longer obtained from natural sources. For example, mannitol and sorbitol are obtained by the hydrogenation of sugars, through Raney nickel catalysts [72].

1.3.19 Leavening Agents

Leavening agents are largely classified into three categories: chemical, biological, and mechanical [73]. The chemicals agents are mainly used at industrial levels. Baking soda is a typical chemical agent which releases carbon dioxide, reacting with heat or moisture [73]. Biological agents digest sugars to produce carbon dioxide through a fermentation process. In this category, baker's yeast (the trade name of the yeast strain from the species Saccharomyces cerevisiae) is the primary leavening agent in breadmaking, especially for industrial bakeries, due to its technological properties [73, 74]. Mechanical agents have rather simple mechanisms, as they release gas that is trapped in the dough. The egg is an example of a mechanical agent, in this case, beaten egg whites [73].

1.3.20 Preservatives

Preservatives are substances used to prevent undesirable changes in foods, prolonging their shelf life and protecting them against deterioration caused by microorganisms [75, 76]. Furthermore, these additives can be used to increase or maintain the nutritional value of foods or to enhance quality and reduce wastage, as well as to enhance consumer acceptability. Some of the commonly used preservatives such as nitrate, and salt have been used for centuries in processed meats and wine [77].

Preservatives are classified according to their origin into natural and synthetic. Natural preservatives include food preservatives obtained from nature, such as salt, sugar, vinegar, spices, honey, and edible oils. Synthetic preservatives include food preservatives that are chemical, semi-synthetic or synthetic in nature, such as benzoates, sorbates, nitrites and nitrates of potassium, sulfites, glutamates, and glycerides. Chemical preservatives are of great importance to the meat industry [76, 77]. The importance of natural preservative compounds is increasing due to the more extensive use of such compounds in food rather than synthetic compounds [75].

1.3.21 Propellants

Propellants are substances that promoted the expansion of foodstuffs. They are closely related to food products, and therefore considered FAs. These compounds are classified according to their physical state (liquids and gaseous compounds), and their use depends on the physiochemical properties of the manufactured food [6]. Some examples of gas propellants are propane, butane, and dimethyl ether, new products named food grade aerosol sprays [78].

1.3.22 Sequestrants

Sequestrants are chemical compounds whose role is to improve the quality and stability of food products. These compounds form chelate complexes with polyvalent metal ions, especially copper, iron, and nickel, which serve as catalysts in the oxidation of the fats in the food [63].

Metals interfere with foodstuffs, modifying their flavor, smell, and color and, consequently, promoting alterations in the final product stability [6].

1.3.23 Stabilizers

Stabilizers are compounds that enable the homogenous dispersion of two or more immiscible materials, while improving the texture, stabilizing the color and flavor, enhancing the overall quality and acceptability of the food products [79]. Their use is fundamental to prevent the separation of food components, facilitating the preparation of fortified and composite foods using two immiscible materials [79]. Alginate, carrageenan, casein, carboxymethylcellulose sodium salt, xanthan, guar, and locust bean gum are the most common natural food stabilizers [6].

1.3.24 Sweeteners

Sweeteners are sugar substitutes that mimic the sweet taste of sugar but have a negligible impact on energy intake [80, 81]. These sugars tend to have desirable sweetness but are not metabolized in the human body and therefore do not provide calorie intake [82].

Sweeteners can be classified in diverse ways, based on their nutritional value, sweetening power, or even their method of production and/or origin. Currently, the trend is to divide them into two large groups: nutritive versus intensive sweeteners or natural versus synthetic sweeteners [83].

There are many artificial sweeteners, but their use is limited because they are often associated with negative health effects. As a consequence, the search for sugar substitutes from natural sources has led to the discovery of a number of substances that possess an intensely sweet taste or taste-modifying properties [84].

Natural sweeteners provide some caloric value but their contribution to energy intake is negligible in the amounts used. Natural sweeteners are isolated from plant materials, having large amounts of sugar sweet constituents [85]. Some of them are erythritol, miraculin, brazzein, curculin, monatin, pentadin, steviol glycosides, stevioside, rebaudiose A, thaumatin, and so forth [86].

Artificial sweeteners are synthetic substances used to replace sugar during the sweetening process of several food products. In general, the use of artificial

sweeteners depends on the granting of legislative approval, for which individual countries have their regulatory requirements [84]. Six of these sweeteners have been approved as FAs by the FDA, including aspartame, neotame, saccharin, acesulfame-k, sucralose, and advantage [82, 87].

1.3.25 Thickeners

Thickeners are substances that consist of modified starches and gums as their base materials [88]. They are used to disperse, stabilize, or prevent the sedimentation of substances in suspensions. Thickeners most commonly used for this purpose are gums, starches, pectins, and their derivatives [6].

1.4 Synthetic Additives vs. Natural Additives

For a variety of reasons, some consumers might regard the use of FAs, especially synthetic ones, with suspicion; FAs are considered unnatural, unhealthy, or even a public health risk. Nevertheless, communications that have allowed consumers to make informed decisions about FAs should be carefully designed and contain the central topics from a risk-related perspective, as well as from a consumer perspective [89].

Obviously, FAs can bring people great sensory enjoyment and commercial convenience, but they may also cause potential risks to human health [4]. For many years, SFAs have gradually replaced NFAs, and many problems involving the abuse of FAs have come to the fore, for example, additives exceeding the standard, or even toxic additives.

SFAs present numerous opportunities to add into the food matrix, for example, stability to temperature or light. Nonetheless, there is an increasing body of evidence showing that overuse of SFAs can considerably increase the risk of certain chronic and acute disorders in human health, including cancer [90]. Many studies have confirmed that the excessive consumption of SFAs is related to gastrointestinal, respiratory, dermatological, and neurological adverse reactions [91–93].

Normally, for the approval of new FAs, intensive risk assessments, usually based on animal studies, are undertaken and FAs already in use are periodically re-evaluated [94]. While a small amount of uncertainty on FAs potential harmfulness cannot be ruled out, food safety experts generally agree on the safety of this approach [94, 95]. Despite differences in adoption across countries, there is a renewed interest in developing safe, natural, and sustainable FAs. Traditionally, these molecules are mainly produced through chemical synthesis or extraction from natural sources.

The synthetic or natural preservatives become part of the food products either directly or indirectly during some phase of their processing, packaging, or storage. Preservation of foodstuffs is necessary to extend the limited durability of foods,

which is related to many factors. Today there are various chemicals used for food preservation but these must first be approved by the responsible food safety authority [96].

Synthetic sweeteners are one of the most widely used FAs in the world, because of their low or even inexistent calories, low cost, and ability to produce a higher sweetness than natural table sugar. Synthetic sweeteners are increasingly being introduced into foods and drinks as sugar substitutes, such as sugar-free desserts and sugar-free sodas [97]. SFAs have been replaced by NFAs in food preservation.

In the last decades, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) have been widely used as FAs to inhibit or delay oxidation. However, considering their possible toxic and carcinogenic effects, the interest of the scientific community has turned towards the recovery of safer antioxidants from natural sources as alternatives to synthetic ones. Such natural antioxidants include phenolic compounds derived from a variety of plant materials such as rosemary, oregano, and sage, among others [98].

Nowadays, a wide array of natural antimicrobials from different sources such as microorganisms, animals, and plants have been researched as potential preservatives. Some examples such as nisin, natamycin [99], lysozyme [100], and the lactoperoxidase system [100, 101] are now used as preservatives in foods and beverages [102]. Other preservatives isolated from *Melissa officinalis*, a plant that has been widely used *Lamiaceae* due to a variety of compounds with biological activities, have been reported in the literature [103]. Aromatic and medicinal plants are good sources of chemical compounds with biological activity [104]. In this way, herbs and spices have been added to different types of foods to improve their flavor and shelf life. It is estimated that 80% of the world's population relies on plant-based traditional medicine [105, 106]. Thus, today's consumers are concerned about the adverse effects of the use of synthetic antimicrobials and appear to prefer foods preserved with natural and safer antimicrobial agents [107]. In connection, the antimicrobial and antioxidant properties attributed to propolis have been applied in the food industry [108].

Nowadays, NFAs are trending in the food industry. One of the most popular NFAs are the antioxidants that have been presented as alternatives to SFAs. Vitamins, polyphenols, and carotenoids are examples of natural antioxidants used in food products [3, 109]. Due to their high antioxidant activity, polyphenols are considered among the most interesting and relevant natural compounds to be used as food preservatives and bioactive ingredients [3, 110, 111].

Most natural compounds cannot be used directly in contact with foods due to their low concentration, high volatility, tasting, and smell. The natural compounds, commonly used as antimicrobial, antifungal, and antioxidant additives in active food packaging are mainly secondary metabolites and essential oils (EOs) isolated from plants and fungi. Natural compounds have potential applications in a wide range of fields such as medicine [112], pharmaceutical, and tissue engineering to develop skin scaffolds [113], agriculture [114, 115], wound healing [116], cosmetic [117], and food packaging [118, 119].

Consumer studies have shown that people have recently become more informed about FAs and always tend to choose additives from the natural origin over their synthetic analogs [3]. This scenario presents a new challenge for food researchers, who have focused their efforts on searching for new natural sources of antioxidant molecules with potential use as FAs [120].

It has now been shown that it is feasible to obtain NFAs for use in the food industry, such as vitamins or carotenoids, which can be used to preserve food. In this way, it has recently been found that there is a greater demand by the food industry for additives of natural origin as an alternative to SFAs, since the latter are associated with toxic effects as above explained. Moreover, the fact that the consumption of functional foods could boost the immune system and resistance of the human body against viruses, has been a priority for consumers over the last decades. This trend has been magnified during the coronavirus (COVID-19) pandemic and is expected to remain high also within the post-lockdown and post-pandemic era [121]. Some sources of natural FAs are *Citrus natsudaidai* peel waste [122], Andean berry [120], Coriander essential oil [123], Yellow Root (*Arcangelisia Flava Merr*) [124] used in the Production Process of Palm Sugar, "kaun" (*trona*) a natural food additive used in Nigeria [125], and pomegranate peel extract [121]..

There is a great deal of curiosity about natural products. This has driven the food industry to create new methods for extracting compounds with natural aromas. Microbial production of food additives can provide advantages over chemical synthesis and natural extraction. These include low-cost starting materials, controllable cultivation processes and product specificity, as well as higher production yields and robustness [126].

Bioconversion is another form of natural synthesis. It is well known that the production of volatile aroma compounds by enzymes or microorganisms for the food industry provides diverse advantages over conventional methods [127]. Plant cell culture is a promising process for the production of flavors and aromas. This method is based on the biochemical, genetic and totipotential capabilities of plant cells [128].

1.5 Adverse Reactions in Humans

FAs are used in foodstuffs to improve the color, texture, taste, extend the food shelf life, acidity regulation, maintain quality, provide fortifying nutrients, and facilitate processing conditions, which makes them a ubiquitous component of the daily life of humans [35, 129, 130].

Although FAs are essential for the storage of foodstuffs, they can give rise to certain health problems. Today's consumers have high-level demands for food safety and express concern about the potential hazards of FAs [129].

Although FAs have regulations governing their use [49], some approved FAs have been associated with potential safety hazards [35]. Different scientific studies have indicated that FAs can cause adverse reactions in humans. Some of the known dangers of FAs are summarized in Table 1.3.

Table 1.3 Some food additives and the reactions that they cause

Functional class	Example	Reported reactions	References
Antioxidant agents	Butylated hydroxyanisole (BHA) (E-320) and butylated hydroxytoluene (E-321)	Increase in the frequency of allergic diseases, hyperactivity, damage to the lungs, liver, and kidneys, and most importantly, urticaria episodes	[131, 132]
Colorings	Carmine (E-120)	Anaphylactic episodes, asthma	[133, 134]
	Tartrazine (E-102)	Allergic asthma	[135]
Flavor enhancers	Monosodium glutamate (MSG) (E-621)	Asthma	[136]
Preservatives	Sodium metabisulphite (E-223)	Contact dermatitis	[137–140]
	Sodium benzoate (E-211)	Hypersensitivity reactions as orofacial granulomatosis, chronic urticaria, anaphylaxis and asthma.	[134, 141, 142]
	Sodium Chloride	High blood pressure, kidney failure, stroke, and heart attack.	[143]
	Benzoic acid (E-210)	Chronic urticaria	[142]

Studies dealing with the adverse reactions of some FAs have reported varied reactions depending on the type of population studied and the criteria. The adverse reactions studied mainly covered skin rashes, respiratory symptoms, gastrointestinal symptoms, and on rare occasions, systemic anaphylaxis [91]. Wilson et al. [91] reviewed a wide variety of symptoms described in the literature and attributed to additive exposure. These symptoms are as follows:

- Dermatological: Angioedema, dermatitis, eczema, flushing, itching, nonspecific rash, sweating, urticaria.
- Gastrointestinal: Abdominal pain, diarrhea, nausea, tongue or throat swelling, vomiting; Respiratory: Asthma exacerbations, cough, rhinitis, shortness of breath, tightness, wheezing.
- Musculoskeletal: Aching, arthralgias, fatigue, myalgias, tightness, weakness.
- Neurological: Behavior disorder, dizziness, fasciculations, headache, migraine, neuropathy, numbness, paresthesias.
- Cardiovascular: Arrhythmias, palpitation, syncope, tachycardia.
- Other: Lacrimation, systemic anaphylaxis, trembling.

1.5.1 Antioxidant Agents

It is scientifically proven that prolonged use of synthetic antioxidants can cause different diseases or physiological disorders, such as asthma, joint pain, dermatitis, and stomach and eye problems; therefore, their use has been restricted [92].

Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are synthetic antioxidant compounds commonly used in many food formulations as food

preservatives due to their antioxidant properties [44]. BHA and BHT have been suspected of inducing adverse reactions in humans, such as urticaria. Goodman et al. [131] reported challenging two patients with chronic idiopathic urticaria, who experienced remissions following dye- and preservative-elimination diets. Both patients noted significant exacerbations of their urticaria after the challenge with BHA and BHT. In another study, Yamaki et al. [132] concluded that BHT might affect allergic diseases Human diseases like allergic rhinitis and asthma. Therefore, BHT might have an impact on allergic diseases in humans.

1.5.2 Colorings

Natural colorants are healthier than artificial ones in many cases. Synthetic colorants may cause allergic reactions and anaphylactic shock in sensitive individuals [48]. Natural colorants are less frequent in hypersensitivity reactions. However, it has been shown that IgE-mediated reactions with carmine and annatto can provoke allergen responses in humans [49]. Another study suggested that people suffering from angioedema and urticaria showed various allergic reactions against carotene and canthaxanthin. A carotene-based dye, annatto, was also reported for anaphylactic shock and the presence of an Annatto-specific IgE antibody was confirmed [144]. Urticaria associated with ingested carmine-colored has also been reported [133].

Synthetic colorants have been also reported to produce severe allergic reactions. The tartrazine used in confectionery, cotton candy, soft drinks, instant puddings, cake mixes, jam, jelly, gelatins, mustard, and many convenience foods, can cause severe allergenic reactions. Several studies also have showed that synthetic colorants may provoke to migraine, blurred vision, itching, rhinitis, suffocation, weakness, heat sensation, palpitation, pruritus, and urticaria [44, 79, 145]. In this context, Gao et al. [146] indicated that tartrazine could cause neurotoxicity and deficits in the learning and memory of mice and rats.

Due to this potential toxicity, it is crucial to control the amount of tartrazine used in food products and it is therefore necessary to develop analytical methods capable of evaluating the exposure of the general population to tartrazine [44].

1.5.3 Flavor Enhancers

Flavoring agents are substances that are chemically defined for use as flavoring. Some flavoring agents are monosodium glutamate, sodium salt of glutamic acid [92]. Monosodium glutamate (MSG) is the flavoring agent that has been most studied for its adverse effects. It is a popular flavor enhancer added to many foods, especially Asian dishes [93]. In 1968, the term ‘Chinese restaurant syndrome’ was coined due to the first clinical reactions produced by MSG.

People sensitive to MSG can experience nausea, breathing problems and other reactions [147]**.** MSG adds extra sodium that can also elevate blood pressure [51].

Furthermore, MSG has been associated with chronic urticaria with angioedema, allergic rhinitis, and bronchial asthma [136, 148].

1.5.4 Preservatives

Preservatives are generally weak organic acids like acetic acid, benzoic acid, citric acid, lactic acid, sorbic acid, and propionic acid [79]. Several studies have shown which preservative agents elicit a variety of adverse reactions, with their use linked to respiratory problems, allergic reactions, anaphylactic shock, and a wide range of other health complications [79, 91].

Benzoates are also suspected of causing asthma, skin rashes, and allergies, while sorbates may cause urticaria and dermatitis [79]. Hypersensitivity reactions to benzoates have been reported since orofacial granulomatosis, chronic urticaria, and bronchial asthma [49, 142]. Similarly, boric acid has been reported to be toxic in humans, suppressing the release of sperm from the testis and reducing fertility by abolishing DNA synthesis in sperm cells [44].

The main FAs associated with hypersensitivity reactions are antioxidants, colorants, and preservatives. FAs are a rare cause of hypersensitivity reactions, in most cases, mild reactions are not mediated by IgE; however, there have been reports of severe reactions (anaphylaxis), and therefore their clinical importance should not be underestimated [76].

1.6 Conclusions

This chapter summarized the importance of using additives in foods, their classification (synthetic and natural food additives) and adverse reactions in humans. In recent years, there has been growing concern about the safety of food additives from a health perspective. Increasingly, consumers are becoming more aware of what they eat, so people need more information about these compounds. Therefore, the use of synthetic food additives requires more awareness and caution, and it is better to substitute them with safe natural substances.

Natural additives are the future of food preservation due to their benefits to health in comparison to synthetics additives. Recently, new products displaying labels of “all natural additives” or “no synthetic additives” have gained increased attention from users. The stability of natural food additives must be investigated aiming to increase the use of these additives in the food industry.

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References

1. Shim SM, Seo SH, Lee Y, Moon GI, Kim MS, Park JH. Consumers' knowledge and safety perceptions of food additives: evaluation on the effectiveness of transmitting information on preservatives. Food Control. 2011;22(7):1054–60.
2. Seo K, Yang Y, Cho B, Gang G, Kim J, Kim E, et al. Seo Et Al 2008.Pdf. 2008. p. 142–8.
3. Carocho M, Morales P, Ferreira ICFR. Natural food additives: quo vadis? Trends Food Sci Technol. 2015;45(2):284–95.
4. Wu L. Analysis of food additives. In: Galanakis CM, editor. Innovative food analysis. London: Academic Press; 2021. p. 157–80.
5. FAO/WHO Codex Alimentarius Commission. General standards for food additives, codex stan 192–1995. Rome: Food Agric Organ United Nations; 2015.
6. Martins FCOL, Sentanin MA, De Souza D. Analytical methods in food additives determination: compounds with functional applications. Food Chem [Internet]. 2019;272:732–50.
7. Saltmarsh M, Insall L. Food additives and why they are used in food. In: Saltmarsh M, editor. Essential guide to food additives. 4th ed. Cambridge: Royal Society of Chemistry; 2013. p. 50–55.
8. Ismail BB, Fuchs R. States Food and Drug Administration (FDA wholesome and appealing food products, 2012). There is a growing use of food. 2020.
9. Blekas GA. Food additives: classification, uses and regulation. In: Encycl Food Heal. Elsevier. Academic press. 2016. p. 731–6.
10. Marinovich M, Galli CL, Bosetti C, Gallus S, La Vecchia C. Aspartame, low-calorie sweeteners and disease: regulatory safety and epidemiological issues. Food Chem Toxicol. 2013;60:109–15.
11. Lotfi A, Bryan N. Role of sodium nitrite curing of meat in curing human disease. In: Reference module in food science. 2022. Available from: https://doi.org/10.1016/B978-0-323-85125-1.00006-5.
12. Quines CB, Rosa SG, Da Rocha JT, Gai BM, Bortolatto CF, Duarte MMMF, et al. Monosodium glutamate, a food additive, induces depressive-like and anxiogenic-like behaviors in young rats. Life Sci. 2014;107(1–2):27–31.
13. Zhang H, Patel J, Bhunia K, Al-Ghamdi S, Sonar CR, Ross CF, et al. Color, vitamin C, β-carotene and sensory quality retention in microwave-assisted thermally sterilized sweet potato puree: effects of polymeric package gas barrier during storage. Food Packag Shelf Life. 2019;21:100324.
14. Saltmarsh M. Chapter 1 Food additives and why they are used. In: Saltmarsh M, editor. Saltmarsh's essential guide to food additives. Cambridge: Royal Society of Chemistry; 2021. p. 1–9.
15. Bearth A, Hartmann C. Consumers' perception and acceptance of food additives. In: Reference module in food science. Elsevier. Academic press. 2017.
16. Minamiyama Y, Takemura S, Ichikawa H. Food additive-induced oxidative stress in rat male reproductive organs and hippocampus. Arch Biochem Biophys. 2021;701:108810.
17. Boutillier S, Fourmentin S, Laperche B. Food additives and the future of health: an analysis of the ongoing controversy on titanium dioxide. Futures. 2020;122:102598.
18. Felter SP, Zhang X, Thompson C. Butylated hydroxyanisole: carcinogenic food additive to be avoided or harmless antioxidant important to protect food supply? Regul Toxicol Pharmacol. Academic Press Inc. 2021;121:104887.
19. Alimohammadi A, Moosavy MH, Amin Doustvandi M, Baradaran B, Amini M, Mokhtarzadeh A, et al. Sodium metabisulfite as a cytotoxic food additive induces apoptosis in HFFF2 cells. Food Chem. 2021;358(March):129910. Available from: https://doi.org/10.1016/j.foodchem.2021.129910.
20. Mcbride DL. Hot topics department safety concerns about food additives and children's health ☆. 2018. Available from: https://doi.org/10.1016/j.pedn.2018.09.008.

21. Cox SR, Sandall AM. Food-additive emulsifiers: the worst thing since sliced bread? Lancet Gastroenterol Hepatol. 2021;6(7):532.
22. Schiano C, Grimaldi V, Scognamiglio M, Costa D, Soricelli A, Nicoletti GF, et al. Soft drinks and sweeteners intake: possible contribution to the development of metabolic syndrome and cardiovascular diseases. Beneficial or detrimental action of alternative sweeteners? Food Res Int. Elsevier Ltd. 2021;142:110220.
23. Bueno-Hernández N, Vázquez-Frías R, Abreu y Abreu AT, Almeda-Valdés P, Barajas-Nava LA, Carmona-Sánchez RI, et al. Review of the scientific evidence and technical opinion on noncaloric sweetener consumption in gastrointestinal diseases. Rev Gastroenterol Méx (Engl Ed). 2019;84(4):492–510.
24. Christofides EA. Artificial sweeteners and obesity—not the solution and potentially a problem. Endocr Pract. 2021;27(10):1052–5.
25. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature [Internet]. 2014;514(7521):181–6. Available from: https://doi.org/10.1038/nature13793.
26. Murray S, Tulloch A, Criscitelli K, Avena NM. Recent studies of the effects of sugars on brain systems involved in energy balance and reward: relevance to low calorie sweeteners. Physiol Behav [Internet]. 2016;164:504–8. Available from: https://doi.org/10.1016/j.physbeh.2016.04.004.
27. Pepino MY. Metabolic effects of non-nutritive sweeteners. Physiol Behav [Internet]. 2015;152:450–5. Available from: https://doi.org/10.1016/j.physbeh.2015.06.024.
28. Mattes RD, Tan S-Y, Tucker RM. Sweeteners: sensory properties, digestion, consumption trends, and health effects. In: Reference module in food science [Internet]. 2021 Jan 1 [cited 2022 Jan 19]. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128218488000147.
29. Abiega-Franyutti P, Freyre-Fonseca V. Chronic consumption of food-additives lead to changes via microbiota gut-brain axis. Toxicology. Elsevier Ireland Ltd. 2021;464:153001.
30. Chassaing B, Koren O, Goodrich JK, Poole AC, Srinivasan S, Ley RE, et al. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. Nature. 2015;519(7541):92–6.
31. Paula Neto HA, Ausina P, Gomez LS, Leandro JGB, Zancan P, Sola-Penna M. Effects of food additives on immune cells as contributors to body weight gain and immune-mediated metabolic dysregulation. Front Immunol. Frontiers Media S.A. 2017;8:1478.
32. Roca-Saavedra P, Mendez-Vilabrille V, Miranda JM, Nebot C, Cardelle-Cobas A, Franco CM, et al. Food additives, contaminants and other minor components: effects on human gut microbiota—a review. J Physiol Biochem. Springer Netherlands. 2018;74:69–83.
33. Trasande L, Shaffer RM, Sathyanarayana S. Food additives and child health [Internet]. 2018. Available from: http://publications.aap.org/pediatrics/article-pdf/142/2/e20181408/1064574/peds_20181408.pdf.
34. Gunathilake T, Akanbi TO, Van Vuong Q, Scarlett CJ, Barrow CJ. Enzyme technology in the production of flavors and food additives. In: Value-addition in food products and processing through enzyme technology [Internet]. 2022 Jan 1 [cited 2022 Jan 19]. p. 45–55. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780323899291000160.
35. Zhang D, Cheng X, Sun D, Ding S, Cai P, Yuan L, et al. AdditiveChem: a comprehensive bioinformatics knowledge-base for food additive chemicals. Food Chem [Internet]. 2020;308:125519. Available from: https://doi.org/10.1016/j.foodchem.2019.125519.
36. Ginex T, Spyrakis F, Cozzini P. FADB: a food additive molecular database for in silico screening in food toxicology. Food Addit Contam – Part A Chem Anal Control Expo Risk Assess. 2014;31(5):792–8.
37. D'souza C, Fernandes R, Kudale S, Naik AS. Local indigenous fruit-derived juices as alternate source of acidity regulators. J Sci Food Agric. 2018;98(5):1995–2001.
38. Han T-Y, Yang Z, Yu C, Xing J-J, Guo X-N, Zhu K-X. Effect of acidity regulators on the shelf life, quality, and physicochemical characteristics of fresh wet noodles. J Cereal Sci

[Internet]. 2021;103(November 2021):103409. Available from: https://doi.org/10.1016/j.jcs.2021.103409.
39. Nurhadi B, Roos YH. Influence of anti-caking agent on the water sorption isotherm and flowability properties of vacuum dried honey powder. J Food Eng [Internet]. 2017;210:76–82. Available from: https://doi.org/10.1016/j.jfoodeng.2017.04.020.
40. Awuchi CG, Twinomuhwezi H, Victory IS, Ikechukwu AO. Food additives and food preservatives for domestic and industrial food applications. J Anim Health. 2020;2(1):1–16.
41. Yapici E, Karakuzu-Ikizler B, Yucel S. Anticaking additives for food powders. In: Ermis E, editor. Food powders properties and characterization. Cham: Springer Nature Switzerland AG; 2021. p. 109–23.
42. Ribeiro JS, Santos MJMC, Silva LKR, Pereira LCL, Santos IA, da Silva Lannes SC, et al. Natural antioxidants used in meat products: a brief review. Meat Sci [Internet]. 2019;148(October 2018):181–8. Available from: https://doi.org/10.1016/j.meatsci.2018.10.016.
43. Aklakur M. Natural antioxidants from sea: a potential industrial perspective in aquafeed formulation. Rev Aquac. 2018;10(2):385–99.
44. Inetianbor J, Yakubu J, Ezeonu SC. Effects of food additives and preservatives on man-a review. 2015. Available from: http://www.journalajst.com.
45. Farr JP, Smith WL, Steichen DS. Bleaching agents. In: Othmer Encycl Chem Technol [Internet], vol 4. 2003. p. 1421–6. Available from: http://www.journalajst.com.
46. Carolina G, Rodica S, Turcanu D, Eugenia C, Olga G. Walnut meal whitening and the impact of whitening factors on its quality. Food Nutr Sci. 2021;12(01):1–12.
47. Clemens R, Pressman P. Coloring the truth: color additives in nutrition and health. Nutr Today. 2018;53(4):169–73.
48. Ramesh M, Muthuraman A. Flavoring and coloring agents: health risks and potential problems [Internet]. In: Natural and artifiial flavoring agents and food dyes: handbook of food bioengineering, vol. 7. Elsevier Inc.; 2018. p. 1–28. Available from: https://doi.org/10.1016/B978-0-12-811518-3/00001-6.
49. Velázquez-Sámano G, Collado-Chagoya R, Cruz-Pantoja RA, Velasco-Medina AA, Rosales-Guevara J. Hypersensitivity reactions to food additives Reacciones de hipersensibilidad a aditivos alimentarios. Resumen Abreviaturas y siglas. 2019;66(3):329–39.
50. Merinas-Amo R, Martínez-Jurado M, Jurado-Güeto S, Alonso-Moraga Á, Merinas-Amo T. Biological effects of food coloring in in vivo and in vitro model systems. Foods. 2019;8(5):176.
51. Svyatelik K. Use of food additives: classification and danger. 1375. p. 123–7.
52. Abdulmumeen HA, Risikat AN, Sururah AR. Food: its preservatives, additives and applications. Int J Chem Biochem Sci. 2012;1(January):36–47.
53. Cox S, Sandall A, Smith L, Rossi M, Whelan K. Food additive emulsifiers: a review of their role in foods, legislation and classifications, presence in food supply, dietary exposure, and safety assessment. Nutr Rev. 2021;79(6):726–41.
54. Pal A, Mondal MH, Adhikari A, Bhattarai A, Saha B. Scientific information about sugar-based emulsifiers: a comprehensive review. RSC Adv. 2021;11(52):33004–16.
55. Marhamati M, Ranjbar G, Rezaie M. Effects of emulsifiers on the physicochemical stability of oil-in-water nanoemulsions: a critical review. J Mol Liq [Internet]. 2021;340:117218. Available from: https://doi.org/10.1016/j.molliq.2021.117218.
56. Mcclure DD, Lamy M, Black L, Kavanagh JM, Barton W. An experimental investigation into the behaviour of antifoaming agents. Chem Eng Sci. 2017;160(October 2016):269–74.
57. Mozuraityte R, Berget I, Mahdalova M, Grønsberg A, Øye ER, Greiff K. Sodium reduction in processed cheese spreads and the effect on physicochemical properties. Int Dairy J [Internet]. 2019;90:45–55. Available from: https://doi.org/10.1016/j.idairyj.2018.10.008.
58. Prosapio V, Norton I. Simultaneous application of ultrasounds and firming agents to improve the quality properties of osmotic PLUS_SPI freeze-dried foods. Lwt [Internet]. 2018;96(March):402–10. Available from: https://doi.org/10.1016/j.lwt.2018.05.068.

59. Vasilaki A, Panagiotopoulou E, Koupantsis T, Katsanidis E, Mourtzinos I. Recent insights in flavor-enhancers: definition, mechanism of action, taste-enhancing ingredients, analytical techniques and the potential of utilization. Crit Rev Food Sci Nutr [Internet]. 2021;0(0):1–17. Available from: https://doi.org/10.1080/10408398.2021.1939264.
60. Rocha RAR, Ribeiro MN, Silva GA, Rocha LCR, Pinheiro ACM, Nunes CA, et al. Temporal profile of flavor enhancers MAG, MSG, GMP, and IMP, and their ability to enhance salty taste, in different reductions of sodium chloride. J Food Sci. 2020;85(5):1565–75.
61. Cavalcante MD, Mauro MA. Aplicação De Agentes Espumantes Veganos Para a Formação De Espumas Na Polpa De Acerola E Açaí / application of vegan foaming agents for the formation of foams in acerola and Açaí pulp. Braz J Dev. 2020;6(10):76702–10.
62. Rodríguez Patino JM, Carrera Sánchez C, Rodríguez Niño MR. Implications of interfacial characteristics of food foaming agents in foam formulations. Adv Colloid Interf Sci. 2008;140(2):95–113.
63. Pandey RM, Upadhyay KU. Food additive. In: El-Samragy Y, editor. Food additive. Rijeka: Intechopen; 2012. p. 1–30.
64. Irkin R, Degirmencioglu N, Guldas M. Effects of organic acids to prolong the shelf-life and improve the microbial quality of fresh cut broccoli florets. Qual Assur Saf Crop Foods. 2015;7(5):737–45.
65. Pérez-Díaz IM. Preservation of acidified cucumbers with a combination of fumaric acid and cinnamaldehyde that target lactic acid bacteria and yeasts. J Food Sci. 2011;76(7):3–7.
66. Ilica RA, Kloetzer L, Galaction AI, Caşcaval D. Fumaric acid: production and separation. Biotechnol Lett. 2019;41(1):47–57.
67. Banerjee S, Bhattacharya S. Food gels: gelling process and new applications. Crit Rev Food Sci Nutr. 2012;52(4):334–46.
68. Park JJ, Olawuyi IF, Park GD, Lee WY. Effects of gelling agents and sugar substitutes on the quality characteristics of carrot jelly. Korean J Food Preserv. 2021;28(4):469–79.
69. Abid M, Yaich H, Hidouri H, Attia H, Ayadi MA. Effect of substituted gelling agents from pomegranate peel on colour, textural and sensory properties of pomegranate jam. Food Chem [Internet]. 2018;239:1047–54. Available from: https://doi.org/10.1016/j.foodchem.2017.07.006.
70. Singh S, Ranjan R, Kumar GA. Role of exudates in food system: a mini-review. J Food Agric Res. 2021;1(1):109–21.
71. Joshi A, Sagar VR, Sharma S, Singh B. Potentiality of potato flour as humectants (anti-staling agent) in bakery product: muffin. Potato Res. 2018;61(2):115–31.
72. Awuci CG, Echeta KC. Current developments in sugar alcohols: chemistry, nutrition, and health concerns of sorbitol, xylitol, glycerol, arabitol, inositol, maltitol, and lactitol. Int J Adv Acad Res. 2019;5(11):2488–9849.
73. Lee G. Change in height of breads baked by different type of leavening agents. Expedition. 2020;10:1-10.
74. Taglieri I, Macaluso M, Bianchi A, Sanmartin C, Quartacci MF, Zinnai A, et al. Overcoming bread quality decay concerns: main issues for bread shelf life as a function of biological leavening agents and different extra ingredients used in formulation. A review. J Sci Food Agric. 2021;101(5):1732–43.
75. Gokoglu N. Novel natural food preservatives and applications in seafood preservation: a review. J Sci Food Agric. 2019;99(5):2068–77.
76. Efenberger-Szmechtyk M, Nowak A, Czyzowska A. Plant extracts rich in polyphenols: antibacterial agents and natural preservatives for meat and meat products. Crit Rev Food Sci Nutr [Internet]. 2021;61(1):149–78. Available from: https://doi.org/10.1080/10408398.2020.1722060.
77. Dwivedi S, Prajapati P, Vyas N, Malviya S, Kharia A. A review on food preservation: methods, harmful effects and better alternatives. Asian J Pharm Pharmacol. 2017;3(6):193–9.
78. Varlet V, Smith F, Augsburger M. New trends in the kitchen: propellants assessment of edible food aerosol sprays used on food. Food Chem [Internet]. 2014;142:311–7. Available from: https://doi.org/10.1016/j.foodchem.2013.07.036.

79. Kumar A, Kumari P, Gupta K, Singh M, Tomer V. Recent advances in extraction, techno-functional properties, food and therapeutic applications as well as safety aspects of natural and modified stabilizers. Food Rev Int [Internet]. 2021;00(00):1–44. Available from: https://doi.org/10.1080/87559129.2021.1950174.
80. Ruiz-Ojeda FJ, Plaza-Díaz J, Sáez-Lara MJ, Gil A. Effects of sweeteners on the gut microbiota: a review of experimental studies and clinical trials. Adv Nutr. 2019;10:S31–48.
81. Mortensen A. Sweeteners permitted in the European Union: safety aspects. Scand J Food Nutr. 2006;50(3):104–16.
82. Chattopadhyay S, Raychaudhuri U, Chakraborty R. Artificial sweeteners – a review. J Food Sci Technol. 2014;51(4):611–21.
83. Elzbieta W. Sweeteners as sugar substitutes in food industry – conditions of use and consumer. Technol Prog Food Process. 2021;1:186–96.
84. Carniel M, Doring T, De Dea J. Sweeteners and sweet taste enhancers in the food industry. JAMA. 2018;38(2):181.
85. Belščak-Cvitanović A, Komes D, Dujmović M, Karlović S, Biškić M, Brnčić M, et al. Physical, bioactive and sensory quality parameters of reduced sugar chocolates formulated with natural sweeteners as sucrose alternatives. Food Chem. 2015;167:61–70.
86. Kim NC, Kinghorn AD. Sweet-tasting and sweetness-modifying constituents of plants. Stud Nat Prod Chem. 2002;27(PART H):3–57.
87. Castro-Muñoz R, Correa-Delgado M, Córdova-Almeida R, Lara-Nava D, Chávez-Muñoz M, Velásquez-Chávez VF, et al. Natural sweeteners: sources, extraction and current uses in foods and food industries. Food Chem. 2022;370:130991.
88. Kim CY, Yoo B. Rheological characterization of thickened protein-based beverages under different food thickeners and setting times. J Texture Stud. 2018;49(3):293–9.
89. Bearth A, Cousin ME, Siegrist M. The consumer's perception of artificial food additives: influences on acceptance, risk and benefit perceptions. Food Qual Prefer [Internet]. 2014;38:14–23. Available from: https://doi.org/10.1016/j.foodqual.2014.05.008.
90. Lu P, Zhang X, Paula Neto HA, Neto PH, Jgb L, Ausina P, et al. Effects of food additives on immune cells as contributors to body weight gain and immune-mediated metabolic dysregulation. Artic 1478 1 Rev [Internet]. 2017;8:1478. Available from: www.frontiersin.org.
91. Wilson BG, Bahna SL. Adverse reactions to food additives. Ann Allergy Asthma Immunol [Internet]. 2005;95(6):499–507. Available from: https://doi.org/10.1016/S1081-1206(10)61010-1.
92. Carocho M, Barreiro MF, Morales P, Ferreira ICFR. Adding molecules to food, pros and cons: a review on synthetic and natural food additives. Compr Rev Food Sci Food Saf. 2014;13(4):377–99.
93. Randhawa S, Bahna SL. Hypersensitivity reactions to food additives. Curr Opin Allergy Clin Immunol. 2009;9:278–83.
94. Emerton V, Choi E. Food additives and why they are used. In: International LF, editor. Essential guide to food additives. Royal society of chemistry. 3rd ed. 2008. p. 1–21.
95. Carrington CD, Bolger PM. The limits of regulatory toxicology. Toxicol Appl Pharmacol. 2010;243(2):191–7.
96. Angiolillo L, Conte A, Del Nobile MA. Food additives: natural preservatives. Encycl Food Saf. 2014;2:474–6.
97. Tandel KR. Sugar substitutes: health controversy over perceived benefits. J Pharmacol Pharmacother. 2011;2(4):236–43.
98. Berdahl DR, Nahas RI, Barren JP. Synthetic and natural antioxidant additives in food stabilization: current applications and future research [Internet]. In: Oxidation in foods and beverages and antioxidant applications: understanding mechanisms of oxidation and antioxidant activity. Woodhead Publishing Limited; 2010. p. 272–320. Available from: https://doi.org/10.1533/9780857090447.2.272.
99. Chen XS, Gao Y, Zhen B, Han D, Zhang JH, Mao ZG. Separation and purification of ϵ-poly-l-lysine from fermentation broth. Process Biochem. 2016;51(1):134–41.

100. Zhang W, Rhim JW. Functional edible films/coatings integrated with lactoperoxidase and lysozyme and their application in food preservation. Food Control. 2022;133:108670.
101. Lara-Aguilar S, Alcaine SD. Lactose oxidase: a novel activator of the lactoperoxidase system in milk for improved shelf life. J Dairy Sci. 2019;102(3):1933–42.
102. Delves-Broughton J. Natural antimicrobials as additives and ingredients for the preservation of foods and beverages. In: Baines D, Seal R, editors. Natural food additives, ingredients and flavourings. Oxford: Woodhead Publishing; 2012. p. 127–61.
103. Carvalho F, Duarte AP, Ferreira S. Antimicrobial activity of Melissa officinalis and its potential use in food preservation. Food Biosci. 2021;44:101437.
104. Mabrouki H, Duarte CMM, Akretche DE. Estimation of total phenolic contents and in vitro antioxidant and antimicrobial activities of various solvent extracts of Melissa officinalis L. Arab J Sci Eng [Internet]. 2018;43:3349–57. Available from: https://doi.org/10.1007/s13369-017-3000-6.
105. FAO-Organización de las Naciones Unidas para la Alimentación y la Agricultura. Codex Alimentarius Norma Internacional De Los Alimentos Norma General Para Los Aditivos Alimentarios Codex Stan 192–1995. FAO OMG [Internet]. 2019;53(9):520. Available from: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B192-1995%252FCXS_192s.pdf.
106. World Health Organization. WHO global report on traditional and complementary medicine 2019 [Internet]. World Health Organization; 2019. p. 1–228. Available from: https://apps.who.int/iris/bitstream/handle/10665/312342/9789241515436-eng.pdf?ua=1.
107. Moghimi R, Ghaderi L, Rafati H, Aliahmadi A, Mcclements DJ. Superior antibacterial activity of nanoemulsion of Thymus daenensis essential oil against E. coli. Food Chem [Internet]. 2016;194:410–5. Available from: https://doi.org/10.1016/j.foodchem.2015.07.139.
108. Da Silva FC, Da Fonseca CR, De Alencar SM, Thomazini M, Balieiro JCDC, Pittia P, et al. Assessment of production efficiency, physicochemical properties and storage stability of spray-dried propolis, a natural food additive, using gum Arabic and OSA starch-based carrier systems. Food Bioprod Process. 2013;91(1):28–36.
109. Carocho M, Ferreira ICFR. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol. 2013;51(1):15–25.
110. Caleja C, Barros L, Antonio AL, Ciric A, Barreira JCM, Sokovic M, et al. Development of a functional dairy food: exploring bioactive and preservation effects of chamomile (Matricaria recutita L.). J Funct Foods [Internet]. 2015;16:114–24. Available from: https://doi.org/10.1016/j.jff.2015.04.033.
111. Caleja C, Barros L, Antonio AL, Ciric A, Soković M, Oliveira MBPP, et al. Foeniculum vulgare Mill. As natural conservation enhancer and health promoter by incorporation in cottage cheese. J Funct Foods. 2015;12:428–38.
112. Mathieu V, Chantôme A, Lefranc F, Cimmino A, Miklos W, Paulitschke V, et al. Sphaeropsidin A shows promising activity against drug-resistant cancer cells by targeting regulatory volume increase. Cell Mol Life Sci. 2015;72(19):3731–46.
113. Ambekar RS, Kandasubramanian B. Progress in the advancement of porous biopolymer scaffold: tissue engineering application. Ind Eng Chem Res. 2019;58(16):6163–94.
114. Moeini A, Masi M, Zonno MC, Boari A, Cimmino A, Tarallo O, et al. Encapsulation of inuloxin A, a plant germacrane sesquiterpene with potential herbicidal activity, in β-cyclodextrins. Org Biomol Chem. 2019;17(9):2508–15.
115. Johnson JL, Raghavan V, Cimmino A, Moeini A, Petrovic AG, Santoro E, et al. Absolute configurations of chiral molecules with multiple stereogenic centers without prior knowledge of the relative configurations: a case study of inuloxin C. Chirality. 2018;30(11):1206–14.
116. Moeini A, Cimmino A, Masi M, Evidente A, Van Reenen A. The incorporation and release of ungeremine, an antifungal Amaryllidaceae alkaloid, in poly(lactic acid)/poly(ethylene glycol) nanofibers. J Appl Polym Sci. 2020;137(37):1–13.

117. Kusumawati I, Indrayanto G. Natural antioxidants in cosmetics [Internet]. In: Studies in natural products chemistry, vol 40. 1st ed. Copyright © 2013 Elsevier B.V. All rights reserved; 2013. p. 485–505. Available from: https://doi.org/10.1016/B978-0-444-59603-1.00015-1.
118. Valerio F, Masi M, Cimmino A, Moeini SA, Lavermicocca P, Evidente A. Antimould microbial and plant metabolites with potential use in intelligent food packaging. Nat Prod Res [Internet]. 2018;32(13):1605–10. Available from: https://doi.org/10.1080/14786419.2017.1385018.
119. Masi M, Moeini SA, Boari A, Cimmino A, Vurro M, Evidente A. Development of a rapid and sensitive HPLC method for the identification and quantification of cavoxin and cavoxone in Phoma cava culture filtrates. Nat Prod Res [Internet]. 2018;32(13):1611–5. Available from: https://doi.org/10.1080/14786419.2017.1392950.
120. Alzate-Arbeláez AF, Dorta E, López-Alarcón C, Cortés FB, Rojano BA. Immobilization of Andean berry (Vaccinium meridionale) polyphenols on nanocellulose isolated from banana residues: a natural food additive with antioxidant properties. Food Chem [Internet]. 2019;294(April):503–17. Available from: https://doi.org/10.1016/j.foodchem.2019.05.085.
121. Kaderides K, Kyriakoudi A, Mourtzinos I, Goula AM. Potential of pomegranate peel extract as a natural additive in foods. Trends Food Sci Technol. 2021;115:380–90.
122. Matsuo Y, Akita K, Taguchi H, Fujii S, Yoshie-Stark Y, Araki T. Utilization and evaluation of Citrus natsudaidai peel waste as a source of natural food additives. Food Chem. 2022;373:131464.
123. Šojić B, Pavlić B, Ikonić P, Tomović V, Ikonić B, Zeković Z, et al. Coriander essential oil as natural food additive improves quality and safety of cooked pork sausages with different nitrite levels. Meat Sci. 2019;157:107879.
124. Heryani H, Nugroho A. Study of yellow root (Arcangelisia Flava Merr) as a natural food additive with antimicrobial and acidity-stabilizing effects in the production process of palm sugar. Procedia Environ Sci. 2015;23:346–50.
125. Imafidon KE, Egberanmwen ID, Omoregie IP. Toxicological and biochemical investigations in rats administered "kaun" (trona) a natural food additive used in Nigeria. J Nutr Intermed Metab. 2016;6:22–5.
126. Sun L, Xin F, Alper HS. Bio-synthesis of food additives and colorants-a growing trend in future food. Biotechnol Adv [Internet]. 2021;47:107694. Available from: https://doi.org/10.1016/j.biotechadv.2020.107694.
127. Paulino BN, Sales A, Felipe L, Pastore GM, Molina G, Bicas JL. Recent advances in the microbial and enzymatic production of aroma compounds. Curr Opin Food Sci. 2021;37:98–106.
128. Ayseli MT, Ipek AY. Flavors of the future: health benefits of flavor precursors and volatile compounds in plant foods. Trends Food Sci Technol. 2016;48:69–77.
129. Kang HJ, Kim S, Kim JW. Trends in korean parents' perceptions on food additives during the period 2014–2018. Nutr Res Pract. 2021;15(3):346–54.
130. Kumar N, Singh A, Sharma DK, Kishore K. Toxicity of food additives. In: Singh RL, Monda S, editors. Food safety and human health. 1st ed. London: Academic Press – Elsevier; 2019. p. 67–98.
131. Goodman DL, McDonnel JT, Nelson HS, Vaughan TR, Weber RW. Chronic urticaria exacerbated by the antioxidant food preservatives, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). J Allergy Clin Immunol. 1990;86(4 PART 1):570–5.
132. Yamaki K, Taneda S, Yanagisawa R, Inoue K-i, Takano H, Yoshino S. Enhancement of allergic responses in vivo and in vitro by butylated hydroxytoluene. Toxicol Appl Pharmacol. 2007;223(2):164–72.
133. Katada Y, Harada Y, Azuma N, Hashimoto J, Sacki Y, Tanaka T. Skin sensitization to carmine before onset of systemic allergy to ingested carmine. World Allergy Organ. 2012;2012(February):2012.
134. Acero S, Tabar AI, Alvarez MJ, Garcia BE, Olaguibel JM, Moneo I. Occupational asthma and food allergy due to carmine. Allergy Eur J Allergy Clin Immunol. 1998;53(9):897–901.

135. Ardern K, Ram FSF, Lasserson TJ. Tartrazine exclusion for allergic asthma. Cochrane Database Syst Rev. 2009;(4).
136. Stevenson DD. Glutamate safety in the food supply the safety evaluation of monosodium glutamate. J Nutr. 2000;130(1981):1049–52.
137. Vitaliti G, Guglielmo F, Giunta L, Pavone P, Falsaperla R. Sodium metabisulphite allergy with multiple food and drug hypersensitivities in a five-year-old child: a case report and literature review. Allergol Immunopathol (Madr). 2015;43(1):106–8.
138. García-Gavín J, Parente J, Goossens A. Allergic contact dermatitis caused by sodium metabisulfite: a challenging allergen. A case series and literature review. Contact Dermatitis. 2012;67(5):260–9.
139. Madan V, Walker SL, Beck MH. Sodium metabisulfite allergy is common but is it relevant? Contact Dermatitis. 2007;57(3):173–6.
140. Alimohammadi A, Moosavy M-H, Amin Doustvandi M, Baradaran B, Amini M, Mokhtarzadeh A, et al. Sodium metabisulfite as a cytotoxic food additive induces apoptosis in HFFF2 cells. Food Chem [Internet]. 2021;358:129910. Available from: https://doi.org/10.1016/j.foodchem.2021.129910.
141. Shepherd GL, Jenkins WJ, Alexander J. Asthma exacerbations in patients taking regular salmeterol, or salbutamol for symptoms. Lancet. 1991;337(8754):1424.
142. Giménez-Arnau A. Contact urticaria and the environment. Rev Environ Health. 2014;29(3):207–15.
143. Glover M, Zuber AM, O'Shaughnessy KM. Hypertension, dietary salt intake, and the role of the thiazide-sensitive sodium chloride transporter NCCT. Cardiovasc Ther. 2011;29(1):68–76.
144. Lucas CD, Hallagan JB, Taylor SL. The role of natural color additives in food allergy. Adv Food Nutr Res. 2001;43(C):195–216.
145. Bosso JV, Simon RA. Urticaria, angioedema, and anaphylaxis provoked by food and drug additives. In: Metcalf DD, Sampson HA, Simon RA, editors. Food allergy: adverse reactions to foods and food additives. 4th ed. Oxford: Blackwell Publishing; 2008. p. 340–52.
146. Gao Y, Li C, Shen J, Yin H, An X, Jin H. Effect of food azo dye tartrazine on learning and memory functions in mice and rats, and the possible mechanisms involved. J Food Sci. 2011;76(6):T125–9.
147. Walker R, Lupien JR. The safety evaluation of monosodium glutamate. J Nutr. 2000;130(4 Suppl):1049–52.
148. Williams AN, Woessner KM. Monosodium glutamate "allergy": menace or myth? Clin Exp Allergy. 2009;39(5):640–6.

Chapter 2
Natural Antioxidants

Maria Jaízia dos Santos Alves, Raul Remor Dalsasso, Germán Ayala Valencia, and Alcilene Rodrigues Monteiro

2.1 Introduction

Oxidative deterioration is one of the major factors that cause the expiration date of food, medicine, and nutraceutical products. Oxidation processes can lead to lipid rancidity [1], degradation of proteins [2], pigments degradation, off flavors, and produce toxic products [3, 4].The oxidative problem also includes food storage under freezing temperatures [5, 6]. Thus, nutritional, sensory, and bioactive quality are highly damaged [7, 8]. Antioxidants are fundamental in several applications, to maintain the properties of these products such as foods, medicines, and nutraceutical during their shelf life commercialization, also avoid their early degradation and losses, besides to maintain the equilibrium between consumer and demand without high costs.

Antioxidants have been studied since the 1920s and were fully adopted by the food industry in the 1940s and 1950s [9]. Nowadays, the most frequently antioxidants used are synthetic substances such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ). Those compounds are efficient, cheap, available, and stable [10, 11]. However, they have been related to health risks, causing progressive regulation restrictions on their use [11, 12] and the rejection by the consumers. Consequently, antioxidants from natural sources have been intensely studied and applied in food and medicine products to substitute the use of synthetic antioxidants.

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Natural antioxidants can be obtained from renewable sources, such as plants, micro-organisms, fungi, algae, animals [10], or even waste material [13–16]. Thus, their use also opens the opportunity to apply renewable raw materials, and valorization of abundant waste [13], such as fruits' peels [17] or spent ground coffee [18], which also contributes to the environment by reducing the waste production. In addition, the processed food content natural antioxidants can receive the "clean" labels once some natural antioxidants are source of the vitamins, minerals and enzymes. On the other hand, these additives must be regulated before the use, because they are not totally inoffensive to human health, thus studies such as toxicity are needed, before they are adopted by industries. Natural antioxidants are also useful when synthetics aren't enough to offer the needed protection inside the regulatory limit, as they can be applied in combination, assuring food quality and safety [9].

Although several studies have been published showing the application of the natural antioxidant for food preservation, studies about their safety when consumed by humans, still are necessary [9]. Thus, this field is important for future research approaches and should base the adoption of natural antioxidant molecules in the list of antioxidant agents for food regulations agencies.

Natural antioxidants bring several benefits once can be used as a component of food ingredients or labeled as another type of additives such as flavoring or coloring agents, while acting as preservation agents [10]. However, also, some disadvantages were related. If higher concentrations are needed to reach stability, the sensorial quality may be affected negatively. Other disadvantages those natural antioxidant are: they are unstable, and sometimes aren't available and possible loss during processing. However, several studies to increase the stability and bioavailability have been shown in the literature [7–10].

The encapsulation of natural antioxidants in micro-, nano-structures, or in films, coatings, and composites for food packaging are solutions for some of these issues [19–23]. These technologies can protect the antioxidant and control its release to the food media, avoiding the negative effects on the sensory properties, besides increasing the stability of the antioxidant, and prolonging its activity. Studies of diversification of raw materials and purification are important to increase the availability and quality of natural antioxidants throughout the year [13, 24–28]. Besides, natural antioxidants come from complex media which are affected by the production processes, i.e., pretreatments, drying, and extraction. So, studies on how those processes affect and potentialize the antioxidant activity of the extracts by changing their composition, purification level, and yields are of great relevance, especially with the application of novel techniques [29, 30].

In general, natural and synthetic antioxidants can be classified based on their mechanism of action: primary, secondary, chelators, quenchers, oxygen scavengers, antioxidant regenerators (synergists), and inhibitors of pro-oxidative enzymes [9, 10]. Primary antioxidants neutralize free radicals by donating electrons, such as phenolic antioxidants [31] α-tocopherol (vitamin E). Secondary antioxidants, or reducing agents, neutralize hydroperoxides into more stable non-radical substances.

This group includes phosphites and sulfur compounds. Chelators bind with metals to prevent them from initiating the formation of free radicals. Ascorbic acid, citric acid, phosphoric acid, soy protein, and milk proteins act by this mechanism. Quenchers neutralize high-energy species, such as singlet oxygen and other photo-activated states, diverting that energy into less detrimental paths. Phenols and carotenoids, such as β-carotene, act in this way. Ascorbic acid and carotenoids, such as astaxanthin, can act as oxygen scavengers since they react with the oxygen in the system, stabilizing it. Finally, regenerators or synergists reduce the radicals that are formed when a primary antioxidant donates a hydrogen atom or electron for a free radical, such as ascorbic acid can act when tocopherol converted into tocopheryl radical is reduced. Inhibitors of pro-oxidative enzymes, such as lipoxygenase, include polyphenolic substances.

In this chapter, the main groups of natural antioxidants will be presented and discussed: ascorbic acid, carotenoids, polyphenols, proteins, and peptides. Also, recent studies presenting their sources, extraction methods, and applications will be reviewed.

2.2 Main Natural Antioxidants

2.2.1 Ascorbic Acid

Ascorbic acid (L-ascorbic acid or ascorbate), or vitamin C, is a natural, non-enzymatic antioxidant found in many plant-based products. It is a water-soluble compound that eliminates reactive oxygen and nitrogen species (RONS) and reduces carbon dioxide, consequently protecting against oxidative stress in vivo and in vitro [32]. Thus, ascorbic acid is beneficial for human health [33]. Ascorbic acid is not synthesized by the human body. Therefore, the diet is considered the primary source [34]. As it is the most widespread, ascorbic acid is one of the most used antioxidants in foods and can be used in practically every country. Chemically, ascorbic acid is a cyclic ester with a ketone at the α position, which donates two electrons to neighboring molecules, becoming oxidized, known in this form as dehydroascorbic acid [35].

It is evident from the list of rich sources of ascorbic acid in Table 2.1 that the main determinant of ascorbic acid intake is fruit and vegetable consumption; deficiency is likely in people whose usual fruit and vegetable intake are too low. This nutrient is found in numerous plant foods, including green vegetables, citrus fruits, tomatoes, berries, potatoes. However, it can be lost due to heat processing and prolonged storage when exposed to ambient conditions such as air, moisture, heat, light, and base resulting in loss of its original functions [1]. Aiming to protect ascorbic acid from damage or oxidation and deliver it to specific locations on the human body, encapsulation and controlled release techniques have been developed, which increases the stability of this compound [33–37].

Type and concentration of ascorbic acid of orange fruits and juices are diverse and vary among the cultivar, environmental and agronomical practices, pre and postharvest conditions, and processing characteristics (see Table 2.1).

Regarding the ascorbic acid degradation mechanism, it is generally accepted that ascorbic acid (AA) reacts via two main pathways, the most common being in the presence of oxygen ("aerobic pathway"), which leads to the formation of dehydroascorbic acid (DHAA), which can then follow different modes of degradation [33, 49, 50]. In the absence of oxygen ("anaerobic pathway"), L-ascorbic acid degrades without being oxidized first. Therefore, DHAA is not formed. Based on this mechanism, in addition to the effect of temperature, the roles of oxygen, oxidizing agents and the presence of the catalyst in the mechanisms and kinetics of L-ascorbic acid degradation have also been widely studied [33, 51, 52].

2.2.2 Carotenoids

Carotenoids are terpenoids phytochemicals comprising β-carotene, astaxanthin, lycopene, and lutein. These compounds have shown potential for reducing the risk of cancer, cardiovascular disease, bone, skin, and eye disorder [53]. Further, carotenoids demonstrated antioxidant, antitumoral, and provitamin A activities [23, 54]. Thus, recent studies have applied them as food additives or incorporated in food packaging as coatings or films aiming the reduction of degrading oxidative reactions and incorporating health benefits, such as the immune system [54–58].

These are natural yellow, orange and red pigments in photosynthetic organisms, such as plants, algae, and cyanobacteria [59] and act as photo protectors. They also can be found in some non-photosynthetic archaea, bacteria, fungi, and animals. These phytochemicals can also be extracted from waste material derived from carotenoid-source organisms, such as peels, kernels, stems, and pomace [14, 16, 55, 59–64]. Some of the several sources of carotenoids are listed in Table 2.2.

Carotenoids are lipophilic isoprenoid compounds with a polyene backbone containing a certain number of double bonds. One of the classifications of carotenoids is based on the presence or absence of end rings, being thus cyclic or acyclic carotenoids. A second classification is based on the molecular composition, which can be split into two groups: (I) hydrocarbon carotenoids or carotenes; (II) xanthophylls, which contain oxygen [59, 61]. Some carotenoids, such as lutein and zeaxanthin, act as light filters avoiding the formation of ROS, thus avoiding oxidation processes [59]. The antioxidant activity can also occur due to the quenching of singlet oxygen, which depletes its excess energy as heat. Thus, the oxygen is returned to an unexcited state, and the carotenoid can be reused as an antioxidant [61]. Carotenoids also quench other free radicals, forming carotene radicals. Thus, carotenoids can act as prooxidants in certain circumstances in which those products accumulate or when carotenoid detoxifying mechanisms are compromised [59].

The factors that affect the carotenoids profile and concentration in vegetable-based extracts or foods involve properties of the biomass, cultivation conditions,

Table 2.1 Ascorbic acid sources and extraction methods

Sources	Extraction method	Content	Reference
Currant (*Ribes L.*) and gooseberry (*Ribes uva-crispa L.*)	Solvent extraction (water/methanol (70:30, v/v)) with shaking water bath at 50 °C for 60 min	Gooseberry (8.57–10.74 g/kg) Currant (6.20–11.57 g/kg)	[36]
Doum (*Hyphaene thebaica*)	80% methanol for 24 h at room temperature	1.60–4.97 mg/g	[37]
Blueberry	Solvent extraction (water/methanol in the ratio of 70:30, v/v) at 50 °C for 60 min	3.06–40.29 mg/100 DW	[38]
Fruits in the mountains of southwest Saudi Arabia (*Coccinia grandis* (L.) *Voigt,Diospyros mespiliformisHochst*. Ex A. Dc.,Cissus rotundifolius (L.), Ephedra foeminea Forssk., and Grewia villosa Willd.)	Methanol	*C. grandis* (896.41 µg/100 mg) *D. mespiliformis* (709.52 µg/100 mg) *C. rotundifolius* (612.14 µg/100 mg) *E. foeminea* (339.15 µg/100 mg) *G. villosa* (241.70 µg/100 mg)	[39]
Strawberry	Nano fertilizers	58.9–63.8 mg/100 g	[40]
Fresh orange juice and cold vegetable soup (gazpacho)	Squeezing	Orange juice (56.3 mg/100 mL) Gazpacho (22.6 mg/100 mL)	[41]
Sweet "Navel" orange juice of the and the red-fleshed Cara Cara	High-pressure	Cara Cara juice (50.96 mg/100 mL) Navel juice (60.18 mg/100 mL)	[42]
Wild apple (*Malus* spp.)	Freeze-dried	58–60 mg/100 g DW	[43]
Cajuí (*Anacardium* spp), murici (*Byrsonima crassifolia* (L.) Kunth), pequi (*Caryocar coriaceum* Wittm.), jenipapo (*Genipa americana* L.), mangaba (*Hancornia speciosa* Gomes), bacuri (*Platonia insignis* Mart.), cajá (*Spondias mombin* L.), umbu-cajá (*Spondias bahiensis* P. Carvalho, Van den Berg & M. Machado), umbu (*Spondias tuberosa* Arruda), pitanga (*Eugenia uniflora* L.), araçá (*Psidium sobralianum* Landrum & Proença)	Freeze-dried	0.36–253.92 mg/100 g	[44]
Citrus cultivars	In nature	24.22–74.85 mg/100 g	[45]

(continued)

Table 2.1 (continued)

Sources	Extraction method	Content	Reference
Nanofiltered extract and comparison with acerola juice	Hydrothermal conditions	Feed (1.6 mg/mL) Permeate (0.6 mg/mL) Concentrate (7.9 mg/mL) Juice (9.1 mg/mL)	[46]
Acerola cherry	In nature	Immature fruit (23.86 mg/g FW) Mature one (12.25 mg/g FW)	[47]
Mango (*Mangifera indica*)	Low temperature drying	17.12–43.44 mg/100 g	[48]

DF dried fruit, *FW* fresh weight, *DW* dry weight

and production process parameters [53]. Biomass properties regard the genotype, location of the carotenoid inside the plant, and leaf to fruit ratio. Cultivation conditions include climatic conditions or agronomic factors, soil composition, and fertilizers [53]. The extraction process also affects the quality of extracts, and it can be performed by conventional solvent extraction or alternative methods, such as ultrasound-assisted, microwave-assisted, enzyme-assisted, pressurized liquid, high hydrostatic pressure, or supercritical fluid extractions [53]. The adequate processing method depends on factors such as the desired quality of the extract, expected by-products/waste production, and technical and economic viability.

2.2.3 Polyphenols

Polyphenols are products of the secondary metabolism of plants, which have a defensive action against aggressors [61]. They are found in most plants and derived products, by-products, and waste materials (Table 2.3). They have shown bioactive properties such as antioxidant, anti-tumoral, anti-inflammatory, vascular-protective, and antimicrobial activities [61]. Its content in the plant matrix is influenced by several factors, from the genetic variety of species, climatic conditions, part of the plant, degree of maturation [104]. The abundance, low cost, renewability, food preservation properties, and health maintaining features make them potential substitutes to synthetic antioxidants additives.

Regarding chemical structure, polyphenols are phenolic systems characterized by two or more phenyl rings and at least one hydroxyl substituent [105]. They are a vast group of secondary metabolites that comprises more than 8000 known compounds [106]. This definition comprises a broad range of molecules with different structures and properties so that polyphenols can be classified as flavonoids and non-flavonoids. Flavonoids generally are organized as C6-C3-C6, corresponding with two aromatic rings combined to three carbons to produce an oxygenated heterocycle [107]. Also, they can be subdivided in the function of the number of phenol

Table 2.2 Sources of carotenoids

Source	Carotenoid	Extraction method	Content	Reference
Tomato waste	Lycopene	Enzyme assisted extraction (cellulase or pectinase)	0.48–0.93 mg/g	[65]
Mantis shrimp (*Oratosquilla nepa*)	Astaxanthin	Chloroform: methanol (2:1, v/v) for 2 min, then chloroform with 10% NaCl	1.9–2.8 mg/g of oil	[66]
Ulva spp. (macroalga)	Lutein, β-carotene, neoxanthin, β-cryptoxanthin, violaxanthin, antheraxanthin, and zeaxanthin	Methanol and acetone	0.02–2.8 mg total carotenoid/g FW	[67]
Damask Rose (*Rosa damascena*) petal	Total carotenoids	Acetone and anhydrous sodium sulfate	0.002–0.055 mg/g dry biomass	[68]
Eucheuma denticulatum (red algae)	β-carotene, astaxanthin, β-cryptoxanthin, fucoxanthin, zeaxanthin, lutein	Freeze dried powder extracted by ethanol for 24 h	3.0–87.7 mg/g dry biomass, respectively	[69]
Fungal (*Umbelopsis isabellina*) production in grape pomace	Total carotenoids, β-carotene, lutein	Methanol:ethyl acetate:petroleum ether (1:1:1, v/v/v), and sepated with diethyl ether and saturated NaCl solution	3.5–4.8 mg/g, 52.97–52.32 mg/100 g dry biomass, respectively	[70]
Persimmon	β-cryoptoxanthin, β-carotene, zeaxanthin, lutein, vioxanthin	Extraction in magnesium carbonate and ethyl acetate	0.95–1.5 mg carotenoid/g DF	[71]
Hypnea musciformis (Red seaweed)	Total carotenoids	Acetone 80%	11.37 mg/g	[72]
Scenedesmus bijuga (microalgae)	Neochrome, neoxanthin, violaxanthin, lutein, zeaxanthin, β-carotene, α-carotene, echinenone	Biomass was extracted by ultrasonic assisted extraction in saline a NaCl solution (120 mol/L)	29.82, 149.73, 79.86, 974.45, 18.77, 268.22, 51.72, 25.71 μg/g DW, respectively	[73]

(continued)

Table 2.2 (continued)

Source	Carotenoid	Extraction method	Content	Reference
Parachlorella kessleri (microalgae)	Total carotenoids	Culture in photobioreactor, followed by cell free-drying, ultrasonic assisted extraction with chloroform and water under stirring, and evaporation	0.011–0.030 μg/mL DW	[74]
Paprika oleoresin	Total carotenoid, yellow carotenoid fraction, red carotenoid	Commercial oleoresin	Ni	[75]
Ripe tainong mango	Neoxanthin, violaxanthin, luteoxanthin, mutatoxanthin, zeaxanthin, antheraxanthin, α-cryptoxanthin, β-cryptoxanthin, α-carotene, β-carotene	Ultrasound-assisted extraction with 80% acetone	1.11, 9.05, 2.70 1.38, 1.13, 0.50, 0.89, 1.51, 0.79, and 4.17 mg/kg DF, respectively	[76]
Kocuria palustris	Sarcinaxanthin	Cell culture followed by extraction by ultrasonic disruption, evaporation, saponification with dichloromethane and methanolic KOH (30%) and NaCl	112.48 μg/L	[77]
Orange-fleshed sweet potato	β-carotene, α-carotene	Cooking (boiling, steaming, microwaving, roasting, or frying), followed by solvent extraction with petroleum ether:acetone (80:20, v/v) for 20 min at 40 °C three times and evaporation	32.02–153.48 and 0.57–0.78 μg/g DW, respectively	[78]

(continued)

Table 2.2 (continued)

Source	Carotenoid	Extraction method	Content	Reference
Passion fruit	Total carotenoid, violaxanthin, antheraxanthin, lutein, zeaxanthin, violaxanthin myristate, violaxanthin palmitate, β-carotene, lutein-3′-O-mystirate, lutein dimystirate	Extraction with acetone	38.1–46.6, 1.54–3.20, 1.63–2.21, 8.41–13.17, 2.10–2.97, 1.39–1.47, 1.36–1.49, 12.32–14.90, 1.53–1.78, 1.32–1.44 μg/g respectively	[79]
Haematococcus pruvialis (green microalga)	Total carotenoids, astaxanthin	Ultrasound-assisted extraction with petroleum ether:acetone:water (15:75:10, v/v/v) and extraction	16.13 mg carotenoids/g of DF	[80]
Sea buckthorn berries (*Hippiphae rhamnoides L.*)	Total carotenoids	Solvent extraction with ethanol:hexane (4:3, v/v)	57.54 mg/g of dry extract	[81]
Oxalis corniculata leaves	Violaxanthin, neoxanthin, lutein, phytofluene	Solvent extraction with absolute ethanol	1.3–7.7, 2.6–39, 102.5–131.8, and 8.3–52 μg/g, respectively	[82]

DF dried fruit, *FW* fresh weight, *DW* dry weight, *Ni* not informed

units, substituent groups, and the linkage type between phenol units. The subgroups of flavonoids comprise anthocyanins (cyanidin, petuninin, malvidin, delphinidin, pelargonidin), flavones (apigenin, luteolin, christin, tangeretin), isoflavonols (genistein, curcumin, daidzein), flavanones (naringenin, hesperitin, neohesperitin, eriodictyol), flavonols (quercetin, kaempferol, rutin, myricetin), flavanols (catechin, epicatechin, gallocatechin, epigallocatechin) and chalcones (arbutin, phloretin, phloridzin) [108], in the function of the oxidation state of the central pyran ring [61, 105, 107]. Flavonoids are among the most commonly found phenolic compounds in fruits and vegetables (Table 2.3).

In non-flavonoids, it can be subdivided into tannins, lignans, and stilbenes [108]. The tannins are the prominent and diverse group, giving foods the specific astringency [108]. Also, the tannins are subdivided in hydrolysable (gallotannins, ellagitannis, punicalin, punicalagin), condensed (procyanidin B_2, Proanthocyanidin A_1, Proanthocyanidin A_2, Proanthocyanidin A_3), and complex (acutissimin) [108].

The antioxidant capacity of a polyphenol is a function of the number and position of hydroxyls in the molecule, as these groups are the main ones responsible for single-electron and hydrogen atom transfers [61].

Table 2.3 Sources of flavonoids

Compounds	Sources	Extraction method	Contents	Reference
Flavonols				
Quercetin	Red onion solid waste	80% methanol	150 mg/100 g	[83]
	Apple (*Malus domestica* Borkh cv. Gala)	Acetone/ethanol (1:3)	0.07–0.53 μg/mg DW	[84]
	Cloudy apple juice	Ni	12 mg/L	[85]
	Tea leaves (*Camellia sinensis*)		374.4 mg/kg	[86]
	Two plum cultivars	Extracted in distilled water and drying	0.06–1.94 mg/kg FW	[87]
	Peach	2% formic acid in methanol with ultrasonic treatment	0.35–1.15 mg/kg FW	[88]
	Pitanga	In water using commercial juice extractor	2.67 mg/100 g FW	[89]
	Pistacia lentiscus	Ni	16–37 mg/100 g DW	[90]
	China aster genotypes	80% methanol	0.05–0.69 μg/100 g	[91]
	Bilberry (*Vaccinium myrtillus*)	Reflux of methanol with 0.6 M of HCl for 2 h	13 μg/g	
Rutin	Peach	2% formic acid in methanol with ultrasonic treatment	0.30–5.54 mg/kg FW	[88]
	Acerola (PEA) and jabuticaba (PEJ) fruit	(1:10 v/v) ethanol 80%	(PEA)-1.0 mg/100 mL (PEJ)-104.4 mg/100 mL	[92]
	Garcinia indica.	1% formic acid in methanol	2.19–4.30 μg/g FW 8.75–17.21 μg/g DW	[91]
	China aster genotypes	80% methanol	0.03–0.51 μg/100 g	
	Mandarin peel	Ethanol or water at 60 °C in shaking water bath for 2 h	Water extract (221.90 μg/g of mandarin peel) Ethanol Extract (214.50 μg/g of mandarin peel)	[93]
	Apples	Citric acid solution of 0.5%	1.95–3.59 mg/100 g DM	[94]
Kaempferol	Acerola (PEA) and jabuticaba (PEJ) fruit	(1:10 v/v) ethanol 80%	(PEA)-11.4 mg/100 mL (PEJ)-1.1 mg/100 mL	[92]
	Pitanga	In water using commercial juice extractor	2.15 mg/100 g fruit	[89]

Anthocyanins				
Cyanidin	*Garcinia indica* genotypes	1% formic acid in methanol	4.47–7.08 mg/g FW	[91]
	Tart cherry	Pressing	C3GR (54.66 mg/kg) C3R (57.49–127.68 mg/kg)	[95]
	Bilberry flower (*Vaccinium myrtillus*)	Reflux of methanol with 0.6 M of HCl for 2 h	86 μg/g	[96]
	Blackberry	80% ethanol and 1% HCl repeated twice at 4 °C for 24 h	Ni	[97]
	Black soybean peel			
	Haskap berry			
	Purple cabbage			
	Purple rice			
	Purple sweet potato			
Petudinin	Black plum peel			
	Black soybean peel			
	Grape peel			
	Licium ruthenium			
Delphinidin	Bilberry fruit (*Vaccinium myrtillus*)	Reflux of methanol with 0.6 M of HCl for 2 h		[96]
	Blueberry	80% ethanol and 1% HCl repeated twice at 4 °C for 24 h		[97]
	Black eggplant peel			
	Black plum peel			
	Black soybean peel			[87]
Malvidin	Bilberry fruit (*Vaccinium myrtillus*)			[96]
	Blueberry			[97]
	Black plum peel			
	Grape peel			
	Haskap berry			

(continued)

Table 2.3 (continued)

Peonodin	Grape peel			
	Haskap berry			
	Purple rice			[87]
	Purple sweetpotato			[97]
	Bilberry fruit (*Vaccinium myrtillus*)			[96]
Pelargonidin	Mulberry			[97]
	Purple rice			
Flavanols				
Catechin	Garcinia indica genotypes	1% formic acid in methanol	24.81–31.11 μg/g FW 99.24–124.4 μg/g DW	[91]
	Pistacia lentiscus	100% methanol (5 g: 40 mL)	16–37 mg/100 g DW	[90]
	China aster genotypes	80% methanol	0.38–28.43 μg/100 g	[98]
	Apple	Citric acid solution of 0.5%	1.19–2.09 mg/100 g DW	[94]
Epicatechin	Pistacia lentiscus		0.4–1 mg/100 g DW	[90]
	China aster genotypes	80% methanol	0.28–2.32 μg/100 g	[98]
	Apple	Citric acid solution of 0.5%	18.1–25.4 mg/100 g DW	[94]

Chalcones				
4,2′,4′-trihydroxy-3′-[(2E,5E)-7-methoxy-3,7-dimethyl-2,5-octadienyl] chalcone	*Angelica keiskei*	MeOH at room temperature overnight and evaporation	Ni	[99]
(±)-4,2′,4′-trihydroxy-3′-[(2E)-6-hydroxy-7-methoxy-3,7-dimethyl-2-octenyl]chalcone				
4,2′,4′-trihydroxy-3′-[(2E)-3-methyl-5-(1,3-dioxolan-2-yl)-2-pentenyl]chalcone				
2′,3′-furano-4-hydroxy-4′-methoxychalcone				
(±)-4-hydroxy-2′,3′-(2,3-dihydro-2-methoxyfurano)-4′-methoxychalcone				
Isoliquiritigenin	Nepalese propolis	MeOH and water reflux		[100]
Spinochalcone C	*Aeschynomene fascicularis*	MeOH for 24 h and vaccum evaporation (40 °C)		[101]
Spinochalcone A				
Flavanones				
Naringenin	Garcinia indica	1% formic acid in methanol	1526–6820 μg/g FW 6107–27,280 μg/g DW	[91]
	Orange, Mandarin, Grapefruit	Methanol:water (50:50 v/v) adjusted at pH 2	Orange (0.124 μg/g) Mandarin (Nd) Grapefruit (0.222 μg/g)	[102]

(continued)

Table 2.3 (continued)

Hesperitin	*Garcinia indica*	1% formic acid in methanol	15.26–31.47 μg/g FW 61.03–125.9 μg/g DW	[91]
	China aster genotypes	80% methanol	0.11–0.92 μg/100 g	[98]
	Mandarin peel	Ethanol or water at 60 °C in shaking water bath for 2 h	Water extract (1643.60 μg/g of mandarin peel) Ethanol extract (1346.44 μg/g of mandarin peel)	[93]
Neohesperitin	Citrus bergamia	H_2O:MeOH 70:30 v/v	Albedo (6.66 mg/g FW) Flavedo (4.89 mg/g FW) Juice (0.96 g/L)	[103]
Eriodictyol	Mandarin peel	Ethanol or water at 60 °C in shaking water bath for 2 h	Water extract (31.24 μg/g of mandarin peel) Ethanol extract (31.10 μg/g of mandarin peel)	[93]
Plathymenin	Nepalese propolis	MeOH and water reflux	Ni	[100]

Nd not determined, *Ni* not informed, *DW* dry weight, *FW* fresh weight, *C3GR* cyanidin-3-glucosyl-rutinoside, *C3R* cyanidin-3-rutinoside

The main antioxidant mechanisms of polyphenols are: (I) direct reaction with free radicals, acting as primary antioxidants, and (II) chelating metals, which catalyze oxidation processes, thus acting as secondary antioxidants. The direct neutralization of free radicals ($R^{\cdot}$) can occur through hydrogen atom transfer, generating an RH and a free radical derived from the polyphenol ($ArO^{\cdot}$). Also, it can occur through single-electron transfer, forming an $R-$ and $ArOH^{+}$ which can be converted to $Ar + H_2O$ in the presence of an H atom. Radicals derived from the polyphenols are much more stable than the R. since the free radical can be stabilized by its movement through the molecule [61].

In flavonoids, the main structure responsible for scavenging species derived from nitric oxide (RNOS) is the catechol group located in the B-ring due to its ability to donate H atoms [61].

2.2.4 Proteins and Peptides

Proteins are macro chains of amino acids linked by peptide bonds. They are fundamental nutrients consumed and produced by animals and other heterotrophic organisms. As a food additive, the food industry applies proteins as emulsifying, gelling, and foaming-forming agents [109]. Similarly, peptides are also a source of amino acids in smaller chains and can be chemically synthesized or produced by the hydrolyze of proteins.

Recently, bioactive proteins and peptides derived from plants and animals have been studied with promising results. Antioxidant proteins and peptides can avoid and stop oxidation chain reactions that can lead to aging and diseases in the human body, such as diabetes, Alzheimer's disease, cancer, cardiovascular diseases [110], among others [111–113], and oxidative degradation of food products [113–115]. Thus, these molecules could be used as substitutes for synthetic antioxidants for food preservation, as they are low cost, non-toxic, and potentially environmental-friendly.

The antioxidant activity of peptides and proteins can occur through different mechanisms: (I) scavenge of oxygen and free radicals; (II) chelating of metal ions; (III) inhibition of lipid peroxidation reactions; (IV) activation of antioxidative defense systems in vivo [113, 116]. The properties of antioxidant peptides are mainly affected by: amino acid composition, sequence, and molecular weight [112, 113].

Free radical scavenge may occur by hydrogen atom or single electron transfer. The main mechanism to take place depends upon the structure of the antioxidant molecules and the partition coefficient [113]. Hydrogen transfer can be measured by oxygen radical absorption capacity (ORAC), total free radical capture antioxidant parameters (TRAP), and carotene bleach analysis [113]. And single electron transfer bases the TEAC and DPPH radical assays [113].

Antioxidant activity by metal ions chelation can occur through three ways: (I) peptides donates hydrogen to maintain the original valence of metal elements,

which prevents metal ions from catalyzing other oxidation processes; (II) peptides chelate metal ions with transport function, thus blocking lipid peroxide formation, which is dependent on the activity of metal ions as coenzymes, especially in the Fenton reaction, (III) peptides could complex with the metal ion component of enzymes, so that the catalyst is rendered ineffective, thereby blocking the process of automatic oxidation of the fat [113]. Ferric reducing antioxidant power (FRAP) is used to evaluate the capacity of reducing Fe^{3+}.

The inhibition of lipid peroxidation chain reactions by antioxidant peptides can stem from: (I) the emulsification promoted by hydrophobic amino acids can expose more active sites of the peptides; (II) inhibition of lipases; (III) antioxidant peptides can disperse on the surface of oil molecules, hindering them to contacting oxygen from the air. For measuring this effect, the β-carotene/linoleic acid systems, thiobarbituric acid (TBARS) assay, iron thiocyanate, electron spin resonance, and peroxide value methods can be used.

Antioxidant peptides can be produced from plants [111, 113, 117] algae [118], and animal material [112, 119–122], including by-products or wastes. Animal-based antioxidants derivate from different products and by-products like meat [121], fish [122], whey [123], chicken bones [15], and pig aorta tissues [110]. From plants, these molecules can be produced from materials such as sasha inchi seeds [124], soy [125], quinoa [126], hemp seeds [127], cowpea [128], sweet potato [129], and brown rice [130]. Antioxidant peptides from plant proteins have some advantages as it is environmental-friendly, renewable, and low cost, mainly as they can be produced from by-products or waste. A list of sources of bioactive proteins and peptides is shown in Table 2.4.

The traditional production process of bioactive peptides involves three main steps [112, 113]: (I) Enzymatic hydrolysis of protein material from food matrix; (II) A treatment to increase the production of the desired peptides, such as high pressure, microwave or ultrasound [125]; (III) a purification process, including membrane separation, electrophoresis, or chromatography.

Bioactive peptides are formed after the hydrolysis of proteins. This process can occur naturally. In milk and vegetables, it is promoted by microbial enzymes during fermentation. In meat curing or seeds germination, endogenous proteinases are the catalysts [112]. But the hydrolysis may also be produced in vitro by animal proteinases such as pepsin, trypsin, chymotrypsin, vegetal proteinases like bromelain and papain, or microbial proteinases, such as *Bacillus* proteinases [131]. This method is the most used, as it offers higher control over the release and obtention of the target peptides [116]. Chemical hydrolysis using acid or alkali are also feasible but result in a substantial loss of amino acids and racemization [121].

The hydrolysis process parameters deeply affect the peptide profile and content and include the type of enzyme, temperature, pH, the ratio of enzyme to substrate, and time. Some types of proteases to be applied in this step are animal proteases, including pepsin, trypsin, chymotrypsin, plant proteases such as papain and bromelain, or microbial proteases such as alcalase, neutral proteases, flavourzyme [113, 116].

Table 2.4 Sources and obtention processes of antioxidant proteins and peptides

Source	Processing	Reference
Milk protein concentrate	Two step enzymatic hydrolysis (Alcalase-Flavourzyme; Alcalase-ProteAXH; Alcalase-Protamex; Alcalase-protease)	[123]
Whey protein	Protein extraction and hydrolysis by microbial proteases: acidic fungal protease II, fungal protease 31,000, fungal protease 60,000, or HT proteolytic protease	[123]
Meat myofibrillar and connective tissue	Protein extraction and hydrolysis by microbial proteases: acidic fungal protease II, fungal protease 31,000, fungal protease 60,000, or HT proteolytic protease	[131]
Chicken bone	Chicken bone hydrolysate (papain, neutral protease or trypsin)	[15]
Laver (*Porphyra haitanensis*)	Proteomics analysis and protein-based bioinformatics	[118]
Sacha inchi seets (*Prukenetia volubilis L.*)	Investigation of changes in antioxidant capacities of protein fractions (albumin, globulin, and glutelin) during simulated gastrointestinal digestion (SGID)	[117]
Soy protein	Epigallocatechin gallate (EGCG). Soy protein fibrils formed by ultrasound treatment.	[125]
Hemp seed	Hemp seed protein enzyme-hydrolysate (Protamex, Novozymes, Bagsvaerd, Denmark)	[127]
Cowpea	Cowpea protein hydrolysate by alcalase	[128]
Sweet potato	Sweet potato protein hydrolyzed by a combination of Alcalase and Favourzyme assisted by energy-divergent ultrasound (EDU), energy-gathered ultrasound (EGU), and energy-gathered ultrasound-microwave (EGUM)	[129]
Brown rice	Germinated brown rice and germinated selenium-enriched germinated brown rice GBR	[130]
Quinoa	Quinoa seeds were turn into flour and defatted method and solvent extraction with hexane	[126]

Alternatively, bioactive peptides can be produced by recombinant DNA technology [112] or by the condensation reaction of amino acids. These techniques can attain high efficiency in the formation of the peptides and are interesting for research. But some drawbacks regarding their application, such as higher costs and extensive research and development phase [121].

Antioxidant properties of peptides and proteins can be enhanced by Maillard reactions, a non-enzymatic reaction involving the carbonyl group of a reducing sugar and a free amino group of protein, peptide, or amino acid, generating products such as aldehydes, esters, furans, ketones, pyrazines, organic acids, and melanoidins, potentially enhancing bioactive properties of proteins and peptides [15, 121, 129].

2.3 Food Applications of Natural Antioxidants

Plant extracts with antioxidant activity have a wide application in the food industry. They can be incorporated into various food matrices, including cheeses, meats, bread, cakes, fruits, with the aim of prolonging shelf life by improving nutritional and sensory preservation of many foods [22, 132–136].

Despite their beneficial effects, natural antioxidants are often easily oxidized and sensitive to heat and light, which limits their application in the food industry [135]. In addition, some of these compounds have other limitations, such as unpleasant taste, low availability, and high susceptibility to storage and processing conditions as well as gastrointestinal environments [137]. In this sense, the application of natural antioxidants, in most cases, occurs when incorporated into active packaging, nanomaterials, among others.

Carotenoids are endogenous colorants and antioxidants of food vegetable foods such as vegetable oils [138, 139] and beans [139, 140], but they can also be used as additives. Carotenoids are widely applied in the food industry as ingredients, additives, and nutritional supplements [59, 139, 141]. A list of recent applications of carotenoids in food products is shown in Table 2.5.

Recently, Ordónez-Santos et al. [57] extracted carotenoids from mandarin epicarp by ultrasound-assisted extraction and added them in cake and bread formulations. The total carotenoid content in the extract was 140.7 ± 2.7 mg of β-carotene equivalent/100 of the dry sample. The carotenoid characterization of the cake and bread produced indicated the presence of β-carotene, β-cryptoxanthin, α-carotene, zeaxanthin, and lycopene in the extracts. The colors of crust and crumb of cake and bread were similar to samples added of tartrazine. Unfortunately, there were not evaluated the sensory acceptance of the product and the antioxidant effect of the extract in the food samples.

Bhimjiyani et al. [55] extracted carotenoids from sea buckthorn pomace using ultrasound-assisted extraction and cold-pressed flaxseed oil (*Linum usitatissimun*) as solvent. The optimized of β-carotene was 11.26 mg/L. The authors verified that the free radical scavenging activity of flaxseed oil measured by DPPH assay was superior to enriched oil than for control oil (98%), without the addition of carotenoids (84%), which could indicate a benefit in the oxidative stability. The oxidative stability of the oils was also evaluated at 100 and 110 °C. The induction time of enriched oil was 6.07 and 2.92 h, respectively, while non-enriched oil was 4.11 and 1.58 h, respectively.

Zuluanga et al. [58] extracted carotenoids from bee pollen using high-pressure pre-treatment and applied them to pineapple juice. The optimized contents in the treated pollen of carotenoids and antioxidant capacities measured by FRAP were 781.31 mg of β-carotene equivalents/kg and 496.9 μmol of Trolox/g. In addition, the treated pollen also had phenolic compounds (67.38 mg GAE/g), which possibly contributed to the antioxidant capacity. Pineapple juice with 10% (w/v) showed contents of carotenoids, phenolics, and antioxidant capacity (FRAP) of 86.60 ± 0.35 mg β-carotene equivalents/kg, 20.34 ± 1.08 mg gallic acid equivalents/g,

and 140.30 ± 0.04 μmol Trolox/g, respectively. Comparing, pure pineapple juice showed the absence of carotenoids, 4.0 ± 0.2 mg gallic acid equivalents/g, and 93.3 ± 4.9 μmol Trolox/g. That indicates that pineapple juice with enriched bee pollen may offer health benefits and higher protection against oxidation thus reducing the nutritional and sensory losses through time.

Despite the benefits of carotenoids to food quality, after extraction these compounds are sensitive to heat and light, hydrophobic, and poorly bioavailable. So, to enhance their benefits as additives, some strategies have been applied to overcome these issues, such as incorporation in emulsions, micro, and nanoencapsulation, or incorporation in biofilms and coatings [23, 54, 73, 80, 81, 141, 142, 152].

In this same line, but now with the aim of increasing the shelf life of bread, which is a perishable product due to intermediate moisture (~40%), a_w (0.94–0.97) and pH close to neutrality [135] which leads to the formation of fungi. Deseta et al. [135] obtained nanocomplexes based on egg white protein nanoparticles (EWPn) and bioactive compounds, carvacrol, thymol, and trans-cinnamaldehyde and evaluated their application as edible antifungal coatings on preservative-free bread. After 7 days of storage, they observed that the coated bread with EWPn-THY and EWPn-CAR nanocomplexes showed the lowest counts, being able to delay significantly fungal development after 7 days of storage [135].

Foods rich in unsaturated fatty acids are highly sensitive to deterioration caused by oxidation [153]. This is a particularly common problem in meat and meat-derived products, for example, quail meat. Raw quail meat can be refrigerated for 2 days, and cooked meat can be stored for up to 3 days. Therefore, to increase its shelf life, it is necessary to use an antibacterial/antioxidant packaging system with active polymers [151].

In this sense, Sani et al. [151] developed an active film based on antioxidant/antibacterial (potato starch/apple pectin/microencapsulated *Zataria multiflora* essential oil/zirconium oxide: St/Pec/MEO/ZrO_2) with controlled release ability of *Zataria multiflora* essential oil in order to increase the shelf life of the meat. Microbial analysis showed that the microbial count in all quail meat packages increased during storage, but the increase in microbial count in the control film is greater than in the active films, which indicates the significant effect of encapsulated essential oil and ZrO_2 nanoparticles in increasing the shelf life of quail meat [151].

In another research, mushroom microparticles (FDMMs) to a achieve synergistic antioxidative effect, and curcumin (CCM) and quercetin (QCT) loaded FDMMs were incorporated in cooked beef patties to inhibit lipid oxidation. The results showed that Patties with FDMMs did not present an increase in TBARS value after 12-day storage, with the CCM-QCT-loaded FDMMs treatment showing a lower level than any other treatments. This suggests that lipid oxidation in cooked patties was significantly inhibited with the incorporation of FDMMs, CCM, and QCT, most effectively with the addition of CCM-QCT-loaded FDMMs [133].

Antioxidant peptides are also antioxidants that can be applied as additives, in active coatings and films, or produced directly in food products aiming at the prevention of oxidation processes and nutritional enrichment [113, 115, 121, 154]. The

Table 2.5 Applications for natural antioxidant compounds

Application	Source	Bioactive	Main results	Reference
Flaxseed oil (*Linun usitatissimun*)	Sea buckthorn pomace	Total carotenoids, β-caroten	Carotenoids increased the antioxidant capacity and stability at 100 and 110 °C of the flaxseed oil	[55]
Food packaging (soy protein isolate or soybean oil)	Commercial	β-carotene	High encapsulation efficiency. Slower and more sustained release under heat treatment	[141, 142]
Complex-beewax oleogel	Commercial	β-carotene	Rheological properties of the oleogels were affected in the presence of β-carotene, enhancing the capacity of the oleogels to retain an oil phase within their crystalline network	[141, 143]
Bakery products (cake and bread)	Mandarin epicarp	β-carotene, β-cryptoxanthin, α-carotene, zeaxanthin, lycopene	Carotenoids added color to bread and cake, with the potential to reduce artificial colorants. The antioxidant activity was not evaluated	[57]
Food packaging (Sunflower oil)	Carrot, tomato, and annatto seeds	β-carotene, lycopene, and bixin	Films containing lycopene and β-carotene protected sunflower oil against light while bixin attenuated the peroxidation and storage conditions	[141, 144]
Bee-polen paste and pineapple juice beverage	Bee polen.	Total carotenoids	High pressure pre-treatment increased the antioxidant activity of bee-polen higher than 60%, in comparison to fresh bee-polen	[58]
Food packaging (Chitosan films)	Pine needle extract	Total phenolics and antioxidants	Films for packaging numerous oxygen-sensitive food products	[145]
Food packaging (Starch composite film) in cheese	Potato peel	Polyphenol	Film showed excellent antioxidant activity and significantly reduced the oxidation rate of cheese	[136]

(continued)

Table 2.5 (continued)

Application	Source	Bioactive	Main results	Reference
Chitosan–procyanidin (CS–PC) composite films in cheese	Ni	Procyanidin	CS–PC films had a significant effect on the preservation of cheese; the characteristics of cheese packaged with CS–PC films were obviously better than those of the control groups	[146]
ZnO bionanocomposites based on RE-ZnO nanocomposites in Ras cheese	Roselle calyx (RE) extract	Total phenolics and antioxidants	Chitosan (CS)/guar gum (GG)/RE-ZnO bionanocomposite films enhance the self-life of the coated Ras cheese in comparison to CS/GG film; CS/GG/RE-ZnO bionanocomposite films was improved its chemical, microbiological, and sensorial properties during ripening time in comparing with uncoated cheese	[147]
Pine needle extract (PNE) incorporated in beeswax for cheese	Pine needle extract (PNE)	Antioxidants	Shelf life and sensory evaluation study including microbiological and sensory analysis revealed inhibition of mold growth and good score of texture and appearance with the increase in concentration of PNE	[132]
Application in packaging of fresh rainbow trout fillets	Essential oil of cinnamon (*Cinnamomum verum*)	Polyphenols	The shelf-life of rainbow trout fillets wrapped was extended to 12 days	[148]

(continued)

Table 2.5 (continued)

Application	Source	Bioactive	Main results	Reference
Fresh soft cheese	Grapefruit seed extract (GSE)	Antioxidants	The lag time of *L. monocytogenes* in soft cheese packed with biodegradable polybutylene adipate-co-terephthalate (with GSE was 2.7 times longer than that of the control, even at the abused temperature of 15 °C)	[149]
Brea	Ni	Carvacrol (CAR), thymol (THY) and trans-cinnamaldehyde (CIN)	The coatings had no impact on the physicochemical properties of the bread loaves (moisture, aw, texture, and color); egg white protein nanoparticle (EWPn)-THY and EWPn-CAR nanocomplexes showed higher antifungal efficacy, extending the bread shelf life after 7 days	[135]
Nitrite-free frankfurter-type sausage	Green tea, stinging nettle and olive leaves extracts	Antioxidants	Combinations of 0.2% ε-Polylysine or 1% Chitosan with Mixed Extract were effective to inhibit total viable count, yeasts and molds growth. 1% Chitosan preserved the luminosity of sausages during refrigerated storage	[150]
Quail meat	*Zataria multiflora* essential oil	Antioxidants	The chemical properties of quail meat packaged with active films were kept of encapsulated essential oil and ZrO_2 nanoparticles and there was an increase the shelf life of quail meat	[151]

(continued)

Table 2.5 (continued)

Application	Source	Bioactive	Main results	Reference
Eggless cake	Flaxseed oil	Antioxidants	ω-3 fatty acids rich cake of acceptable quality characteristics can be obtained meeting requirements of vegetarians using nano-encapsulated flax seed oil powder	[22]
Cake	Butcher's broom (*Ruscus Hyrcanus L*) leaves (BBL)	Antioxidants	Adding the nano-capsulated extract of BBL to the cake a reduced the number of spoilage organisms, delayed their oxidations and extend their shelf life	[134]
Beef patties	Ni	Curcumin and quercetin	Mushroom microparticles for incorporating a variety of natural lipophilic antioxidants improved lipid oxidation in cooked beef patties during storage	[133]
Vacuum packed sausages	Garlic essential oil (GEO)	Antioxidants	The active films retarded lipid oxidation and the growth spoilage bacterial groups compared to the control, exhibiting the best result with the peroxide value, thiobarbituric acid reactive substances and aerobic plate count of 0.37 (meq/kg lipid), 0.47 (mg malondialdehyde/kg) and 3.69 (log CFU/g), respectively, on day 50. The GEO made no significant differences in the sensory properties comparing to free-GEO samples ($P < 0.05$)	[137]

Ni not informed, *RE* Roselle calyx, *PNE* pine needle extract, *GSE* grapefruit seed extract, *CAR* carvacrol, *THY* thymol, *CIN* trans-cinnamaldehyde, *BBR* Butcher's broom, *GEO* garlic essential oil

oxidation in food can lead to rancidity, color changes, off-flavors, nutritional losses, and production of toxic substances, so these molecules promote greater shelf lives for food products. Also, as many bioactive peptides can be related to the prevention effect of many non-communicable diseases, and are low-cost, non-toxic, and environmental-friend, their application as food additives can add significant food products [113], as shown in Table 2.5.

Antioxidant proteins and peptides have been intensely researched, and currently, the studies are focused on the identification and determination of pharmacological bioactivities of the molecules in vitro or in vivo. Still, there is a lack of studies regarding the food preserving potential.

El-Saadony et al. [114] applied hen egg isolate, duck egg isolate, pepper seed protein, and pepsin-kidney protein hydrolysate in refrigerated buffalo raw milk. During 30 days, all the additives increased the oxidative stability of the milk. Pepper seed protein and kidney bean protein hydrolysate reduced the decay of sugars in the milk by reducing 45% of bacterial load, compared to other milk samples. Pepper seed protein scavenged 87% of DPPH. Pepper seed protein and kidney beans reduced the growth of viable bacteria, molds, and yeasts.

Przybylksi et al. [115] applied peptides (Thr-Ser-Lys-Tyr-Arg) produced by pepsin hydrolysis from bovine cruor, a slaughterhouse by-product, in ground beef. The results showed that the peptide had similar results to butylated hydroxytoluene (BHT). The extracted peptide reduced the lipid oxidation of the food by about 60% and inhibited the microbial, molds, and yeasts growth during storage refrigeration for 14 days.

Keska et al. [155] investigated the effect of the hydrolysis of proteins from dry-cured pork loins by lactic acid *bacteria Lactobacillus rhamnosus* LOCK 900, *Lactobacillus acidophilus* Bauer L0938, and *Lactobacillus animalis* ssp. lactis BB-12. Results indicated that the type of microorganism did not influence the relative quantity of peptides and that there is no relationship between this parameter and the antioxidant activity. The hydrolysates were able to enhance the antioxidant capacities measured by DPPH, Fe^{2+}, Cu^{2+} in 1.54–7.33, 11.54–125.52, and 5.49–25.24%, respectively compared to unhydrolyzed samples.

Tkaczewska et al. [154] applied antioxidant peptides in biopolymer films for food conditioning. The peptides were produced by the hydrolysis of carp skin gelatin and mixed to furcellaran, an algae polysaccharide, and glycerol to form films by casting. The preservation performance was tested in atlantic mackerel carcasses stored at 4 °C for 15 days. The films reduced the lipid oxidation in the samples, mainly at the early oxidation stages. Also, the coatings inhibited the microbial growth (total viable counts, yeasts and molds, Pseudomonas counts), which resulted in 2 days increase in shelf life. A consumers' acceptance test indicated that the films did not affect the attractiveness of the Atlantic mackerel carcasses.

2.4 Prospects and Limitations of Natural Food Antioxidants

Although the health benefits of carotenoids are hard to confirm in vivo, there is consistent evidence of benefits so far. Also, the positive effects of those molecules in food preservation and sensory enhancement as antioxidants and colorants have already been proved. Some ways to enable their use as substitutes of artificial antioxidants and colorants are: (I) to identify economically viable and sustainable sources, such as by-products and waste materials; (II) to develop microbiological production of carotenoids by microalgae and bacteria cultivations, which could include metabolic engineering and synthetic biology; (III) to study non-traditional extraction processes and treatments, including ultrasound, enzymes, microwave, high pressure, pulsed light, cold plasma, and electric fields, and optimization aiming to reduce the production cost of carotenoids, increasing the yields, while avoiding losses during production; and (IV) to enhance the stability of carotenoids during foods' storage by nanoencapsulation or incorporation in biopolymeric coatings and films, so as the controlled liberation to the food matrices; (V) to study the bioactivity and stability of carotenoids in different food systems using different food models.

The application of antioxidant peptides in food systems is promising. But, much of the effort so far was directed to the production and identification [32, 64–66] of these compounds and their pharmacological effects [112, 113] which is itself a broad field to explore. Enhancing shelf life studies may be simpler and cheaper, although there some issues are limiting the industrial application: (I) lack of studies regarding the effects of antioxidant peptides on oxidation in food models involving the effects over oxidation, shelf life, safety, and sensorial characteristics; (II) These molecules are not still produced in commercial scale, and up-scale studies are needed; (III) purification step can be better explored; (IV) the relationship between the peptides' characteristics and their antioxidant activity is not completely understood.

2.5 Conclusions

In this chapter, the most used and studied natural antioxidants were discussed. As it could be observed, a wide range of diverse molecules presents antioxidant properties. They can be applied as purified compounds, in extracts, or as components of ingredients or raw materials to food products, promoting higher quality, shelf-life, bioactive properties, and antimicrobial activity. Natural antioxidants can show diverse molecular forms and act many mechanisms. Besides, several of them present antimicrobial mechanisms as part of their biological function on the origin organism.

Studies on "natural antioxidants" have been on a progressive rise mainly since the early 1990s (Web of Science database), passing from 1 in 1990 to 545 papers in 2021. This is already reflected in food industry regulations around the world and in

an increase of natural antioxidants used in food products, as replaced by synthetic. Natural antioxidants are naturally present in food products and ingredients, yet they were not added as an additive. However, further research on safety and bioactivity is needed to increase the range of natural antioxidants accepted by governments as food additives.

Despite the benefits of natural antioxidants, some scientific and technological gaps to be elucidated still delay broader employment in the food industry. The major issues are cost reduction, higher availability, enhancement of stability, antioxidant potential, and safety. Studies in those areas, such as encapsulation and controlled liberation, diversification of raw materials, and improvement of pretreatments, production, and purification processes are highly relevant nowadays as in the following years.

With a deeper knowledge of the obtention of these substances in commercially feasible ways, more natural antioxidants will take their part in food products, and consumers will be less worried about consuming synthetic substances. Furthermore, as several antioxidants are obtained from waste or by-products, the production of residues can be reduced and converted to high-value ingredients, which enables the supply of high-quality food products with a long shelf-life. That contributes to better availability of safe food in a more sustainable future.

Conflicts of Interest The author declares no conflict of interest.

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References

1. Waraho T, Mcclements DJ, Decker EA. Mechanisms of lipid oxidation in food dispersions. Trends Food Sci Technol [Internet]. 2011;22(1):3–13. Available from: https://doi.org/10.1016/j.tifs.2010.11.003.
2. Nawaz A, Irshad S, Ali I, Khalifa I, Walayat N, Muhammad R, et al. Protein oxidation in muscle-based products: effects on physicochemical properties, quality concerns, and challenges to food industry. Food Res Int [Internet]. 2022;157(February):111322. https://doi.org/10.1016/j.foodres.2022.111322.
3. Maldonado-Pereira L, Schweiss M, Barnaba C, Medina-Meza IG. The role of cholesterol oxidation products in food toxicity. Food Chem Toxicol [Internet]. 2018;118(May):908–39. Available from: https://doi.org/10.1016/j.fct.2018.05.059.
4. Kubow S. Routes of formation and toxic consequences of lipid oxidation products in foods. Free Radic Biol Med. 1992;12(1):63–81.

5. Mariano BJ, de Oliveira VS, Chávez DWH, Castro RN, Riger CJ, Mendes JS, et al. Biquinho pepper (Capsium chinense): bioactive compounds, in vivo and in vitro antioxidant capacities and anti-cholesterol oxidation kinetics in fish balls during frozen storage. Food Biosci. 2022;47(February):101647.
6. Al-Dalali S, Li C, Xu B. Effect of frozen storage on the lipid oxidation, protein oxidation, and flavor profile of marinated raw beef meat. Food Chem. 2022;376(June 2021):131881. Available from: https://doi.org/10.1016/j.foodchem.2021.131881.
7. Jensen PN, Danielsen B, Bertelsen G, Skibsted LH, Andersen ML. Storage stabilities of pork scratchings, peanuts, oatmeal and muesli: comparison of ESR spectroscopy, headspace-GC and sensory evaluation for detection of oxidation in dry foods. Food Chem. 2005;91(1):25–38.
8. Cichoski AJ, da Silva JS, Leães YSV, Robalo SS, dos Santos BA, Reis SR, et al. Effects of ultrasonic-assisted cooking on the volatile compounds, oxidative stability, and sensory quality of mortadella. Ultrason Sonochem. 2021;72:105443.
9. Berdahl DR, Nahas RI, Barren JP. Synthetic and natural antioxidant additives in food stabilization: current applications and future research. In: Decker E, Elias R, McClements DJ, editors. Oxidation in foods and beverages and antioxidant applications: Volume 1: Understanding mechanisms of oxidation and antioxidant activity. Woodhead Publishing Limited; 2010. p. 272–320. Available from: https://doi.org/10.1533/9780857090447.2.272.
10. Pokorny J. Are natural antioxidants better – and safer – than synthetic antioxidants? Eur J Lipid Sci Technol. 2007;109:629–42.
11. Wang X, Martínez M, Anad A. Synthetic phenolic antioxidants: metabolism, hazards and mechanism of action. Food Chem. 2021;353:129488.
12. Wang W, Xiong P, Zhang H, Zhu Q, Liao C. Analysis, occurrence, toxicity and environmental health risks of synthetic phenolic antioxidants: a review. Environ Res. 2021;201(June):111531. Available from: https://doi.org/10.1016/j.envres.2021.111531.
13. Moure Â, Cruz JM, Franco D, Domõânguez JM, Sineiro J, Domõânguez H, et al. Natural antioxidants from residual sources. Food Chem. 2001;72:145–71.
14. Elik A, Yanık DK, Göğüş F. Microwave-assisted extraction of carotenoids from carrot juice processing waste using flaxseed oil as a solvent. Lebensm Wiss Technol. 2020;123(December 2019):109100.
15. Nie X, Xu D, Zhao L, Meng X. Antioxidant activities of chicken bone peptide fractions and their Maillard reaction products: effects of different molecular weight distributions. Int J Food Prop [Internet]. 2017;20(1):457–66. Available from: https://doi.org/10.1080/10942912.2017.1299176.
16. Poletto P, Álvarez-Rivera G, López GD, Borges OMA, Mendiola JA, Ibáñez E, et al. Recovery of ascorbic acid, phenolic compounds and carotenoids from acerola by-products: an opportunity for their valorization. Lebensm Wiss Technol. 2021;146(May):111654.
17. de Sousa ASB, da Silva MCA, Lima RP, Meireles BRL d A, Cordeiro ATM, Santos EF d S, et al. Phenolic compounds and antioxidant activity as discriminating markers and adding value of mango varieties. Sci Hortic (Amsterdam). 2021;287:110259.
18. Torres-valenzuela LS, Ballesteros-gómez A, Sanin A, Rubio S. Separation and Purification technology valorization of spent coffee grounds by supramolecular solvent extraction. Sep Purif Technol. 2019;228(July):115759. Available from: https://doi.org/10.1016/j.seppur.2019.115759.
19. Teixeira BF, Aranha JB, Maria T, De SF. Replacing synthetic antioxidants in food emulsions with microparticles from green acerola (Malpighia emarginata). Futur Foods. 2022;5(October 2021):100130. Available from: https://doi.org/10.1016/j.fufo.2022.100130.
20. Luiza Koop B, Nascimento da Silva M, Diniz da Silva F, Thayres dos Santos Lima K, Santos Soares L, José de Andrade C, et al. Flavonoids, anthocyanins, betalains, curcumin, and carotenoids: sources, classification and enhanced stabilization by encapsulation and adsorption. Food Res Int. 2022;153(December 2021):110929.
21. Yousefi M, Shadnoush M, Sohrabvandi S, Khorshidian N, Mortazavian AM. Encapsulation systems for delivery of flavonoids: a review. Biointerface Res Appl Chem. 2021;11(6):13934–51.

22. Murugkar DA, Zanwar AA, Shrivastava A. Effect of nano-encapsulation of flaxseed oil on the stability, characterization and incorporation on the quality of eggless cake. Appl Food Res. 2021 Dec;1(2):100025.
23. Rehman A, Tong Q, Jafari SM, Assadpour E, Shehzad Q, Aadil RM, et al. Carotenoid-loaded nanocarriers: a comprehensive review. Adv Colloid Interface Sci [Internet]. 2020;275:102048. Available from: https://doi.org/10.1016/j.cis.2019.102048.
24. Zuorro A. Optimization of polyphenol recovery from espresso coffee residues using factorial design and response surface methodology. Sep Purif Technol [Internet]. 2015;152:64–9. Available from: https://doi.org/10.1016/j.seppur.2015.08.016.
25. del Pozo C, Bartrolí J, Alier S, Puy N, Fàbregas E. Production of antioxidants and other value-added compounds from coffee silverskin via pyrolysis under a biorefinery approach. Waste Manag [Internet]. 2020;109:19–27. Available from: https://doi.org/10.1016/j.wasman.2020.04.044.
26. Xavier Machado T d O, Portugal IBM, Padilha CV d S, Ferreira Padilha F, dos Santos Lima M. New trends in the use of enzymes for the recovery of polyphenols in grape byproducts. J Food Biochem. 2021;45(5):1–13.
27. Singh B, Singh JP, Kaur A, Singh N. Phenolic composition, antioxidant potential and health benefits of citrus peel. Food Res Int. 2020;132:109114.
28. Sun R, Lu J, Nolden A. Nanostructured foods for improved sensory attributes. Trends Food Sci Technol [Internet]. 2021;108(August 2020):281–6. Available from: https://doi.org/10.1016/j.tifs.2021.01.011.
29. Dalsasso RR, Valencia GA, Monteiro AR. Impact of drying and extractions processes on the recovery of gingerols and shogaols, the main bioactive compounds of ginger. Food Res Int. 2022;154(November 2021):111043.
30. Gil-Martín E, Forbes-Hernández T, Alejandro R, Cianciosi D, Giampieri F, Battino M. Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products [Internet]. Food Chem. Elsevier Ltd. 2021;378:131918. Available from: https://doi.org/10.1016/j.foodchem.2021.131918.
31. Zamora R, Hidalgo FJ. The triple defensive barrier of phenolic compounds against the lipid oxidation-induced damage in food products. Trends Food Sci Technol [Internet]. 2016;54:165–74. Available from: https://doi.org/10.1016/j.tifs.2016.06.006.
32. Hoang HT, Moon J-Y, Lee Y-C. Natural antioxidants from plant extracts in skincare cosmetics: recent applications, challenges and perspectives. Cosmetics. 2021;8(4):106.
33. Giannakourou MC, Taoukis PS, Silva M, Miller FA, Ribeiro TM, Brandão S. Effect of alternative preservation steps and storage on vitamin C stability in fruit and vegetable products: critical review and kinetic modelling approaches. Foods. 2021;10(11):2630.
34. Bedhiafi T, Inchakalody VP, Fernandes Q, Mestiri S, Billa N, Uddin S, et al. The potential role of vitamin C in empowering cancer immunotherapy. Biomed Pharmacother. 2022 Feb;146:112553.
35. Carocho M, Morales P, Ferreira ICFR. Antioxidants: reviewing the chemistry, food applications, legislation and role as preservatives. Trends Food Sci Technol. 2018;71:107–20.
36. Orsavová J, Hlaváčová I, Mlček J, Snopek L, Mišurcová L. Contribution of phenolic compounds, ascorbic acid and vitamin E to antioxidant activity of currant (Ribes L.) and gooseberry (Ribes uva-crispa L.) fruits. Food Chem. 2019;284:323–33.
37. Kolla MC, Laya A, Bayang JP, Koubala BB. Effect of different drying methods and storage conditions on physical, nutritional, bioactive compounds and antioxidant properties of doum (Hyphaene thebaica) fruits. Heliyon. 2021;7(4):e06678.
38. Zia MP, Alibas I. Influence of the drying methods on color, vitamin C, anthocyanin, phenolic compounds, antioxidant activity, and in vitro bioaccessibility of blueberry fruits. Food Biosci. 2021;42:101179.
39. Hegazy AK, Mohamed AA, Ali SI, Alghamdi NM, Abdel-Rahman AM, Al-Sobeai S. Chemical ingredients and antioxidant activities of underutilized wild fruits. Heliyon. 2019;5(6):e01874.

40. Rahman MH, Hasan MN, Khan MZH. Study on different nano fertilizers influencing the growth, proximate composition and antioxidant properties of strawberry fruits. J Agric Food Res. 2021;6:100246.
41. Elez-Martínez P, Martín-Belloso O. Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. Food Chem. 2007;102(1):201–9.
42. De Ancos B, Rodrigo MJ, Sánchez-Moreno C, Pilar Cano M, Zacarías L. Effect of high-pressure processing applied as pretreatment on carotenoids, flavonoids and vitamin C in juice of the sweet oranges "Navel" and the red-fleshed "Cara Cara". Food Res Int. 2020;132:109105.
43. Radenkovs V, Püssa T, Juhnevica-Radenkova K, Kviesis J, Salar FJ, Moreno DA, et al. Wild apple (Malus spp.) by-products as a source of phenolic compounds and vitamin C for food applications. Food Biosci. 2020;38:100744.
44. de Assis RC, de Lima Gomes Soares R, Siqueira ACP, de Rosso VV, de Sousa PHM, Mendes AEP, et al. Determination of water-soluble vitamins and carotenoids in Brazilian tropical fruits by high performance liquid chromatography. Heliyon. 2020;6(10):e05307.
45. Coelho EM, da Silva Haas IC, de Azevedo LC, Bastos DC, Fedrigo IMT, dos Santos LM, et al. Multivariate chemometric analysis for the evaluation of 22 citrus fruits growing in Brazil's semi-arid region. J Food Compos Anal. 2021;101:103964.
46. Alves Borges OM, Cesca K, Arend GD, Alvarez-Rivera G, Cifuentes A, Ferreira Zielinski AA, et al. Integrated green-based methods to recover bioactive compounds from by-product of acerola processing. Lebensm Wiss Technol. 2021;151:112104.
47. Xu M, Shen C, Zheng H, Xu Y, Xue C, Zhu B, et al. Metabolomic analysis of acerola cherry (Malpighia emarginata) fruit during ripening development via UPLC-Q-TOF and contribution to the antioxidant activity. Food Res Int. 2020;130:108915.
48. Thi Thanh Huong N, Thinh P, Vinh Long D, Bao Long H, Thanh Dat U, Tan Phat D, et al. Effects of microwave and ultrasound treatment on vitamin C, polyphenols and antioxidant activity of mango (Mangifera indica) during low temperature drying. Mater Today Proc. 2022;59(1):781–6.
49. Vieira MC, Teixeira AA, Silva CLM. Mathematical modeling of the thermal degradation kinetics of vitamin C in cupuaçu (Theobroma grandiflorum) nectar. J Food Eng. 2000;43(1):1–7.
50. Van Bree I, Baetens JM, Samapundo S, Devlieghere F, Laleman R, Vandekinderen I, et al. Modelling the degradation kinetics of vitamin C in fruit juice in relation to the initial headspace oxygen concentration. Food Chem. 2012;134(1):207–14.
51. Fustier P, St-Germain F, Lamarche F, Mondor M. Non-enzymatic browning and ascorbic acid degradation of orange juice subjected to electroreduction and electro-oxidation treatments. Innov Food Sci Emerg Technol. 2011;12(4):491–8.
52. Odriozola-Serrano I, Soliva-Fortuny R, Martín-Belloso O. Influence of storage temperature on the kinetics of the changes in anthocyanins, vitamin C, and antioxidant capacity in fresh-cut strawberries stored under high-oxygen atmospheres. J Food Sci. 2009;74(2):C184–91.
53. Meléndez-Martínez AJ, Mapelli-Brahm P. The undercover colorless carotenoids phytoene and phytofluene: importance in agro-food and health in the Green Deal era and possibilities for innovation. Trends Food Sci Technol. 2021;116(April):255–63.
54. Santos PD d F, Rubio FTV, Balieiro JC d C, Thomazini M, Favaro-Trindade CS. Application of spray drying for production of microparticles containing the carotenoid-rich tucumã oil (Astrocaryum vulgare Mart.). Lebensm Wiss Technol. 2021;143(February):111106.
55. Bhimjiyani VH, Borugadda VB, Naik S, Dalai AK. Enrichment of flaxseed (Linum usitatissimum) oil with carotenoids of sea buckthorn pomace via ultrasound-assisted extraction technique: enrichment of flaxseed oil with sea buckthorn. Curr Res Food Sci. 2021;4:478–88.
56. Mantovani RA, Rasera ML, Vidotto DC, Mercadante AZ, Tavares GM. Binding of carotenoids to milk proteins: why and how. Trends Food Sci Technol [Internet]. 2021;110(January):280–90. Available from: https://doi.org/10.1016/j.tifs.2021.01.088.

57. Ordóñez-Santos LE, Esparza-Estrada J, Vanegas-Mahecha P. Ultrasound-assisted extraction of total carotenoids from mandarin epicarp and application as natural colorant in bakery products. Lebensm Wiss Technol. 2021;139(September 2020):110598.
58. Zuluaga C, Martínez A, Fernández J, López-Baldó J, Quiles A, Rodrigo D. Effect of high pressure processing on carotenoid and phenolic compounds, antioxidant capacity, and microbial counts of bee-pollen paste and bee-pollen-based beverage. Innov Food Sci Emerg Technol [Internet]. 2016;37:10–7. Available from: https://doi.org/10.1016/j.ifset.2016.07.023.
59. Rodriguez-Concepcion M, Avalos J, Bonet ML, Boronat A, Gomez-Gomez L, Hornero-Mendez D, et al. A global perspective on carotenoids: metabolism, biotechnology, and benefits for nutrition and health. Prog Lipid Res. 2018;70(April):62–93.
60. Gómez-García R, Campos DA, Oliveira A, Aguilar CN, Madureira AR, Pintado M. A chemical valorisation of melon peels towards functional food ingredients: bioactives profile and antioxidant properties. Food Chem [Internet]. 2021;335(July 2020):127579. Available from: https://doi.org/10.1016/j.foodchem.2020.127579.
61. Gutiérrez-Del-río I, López-Ibáñez S, Magadán-Corpas P, Fernández-Calleja L, Pérez-Valero Á, Tuñón-Granda M, et al. Terpenoids and polyphenols as natural antioxidant agents in food preservation. Antioxidants. 2021;10(8):1264.
62. Marçal S, Pintado M. Mango peels as food ingredient / additive: nutritional value, processing, safety and applications. Trends Food Sci Technol [Internet]. 2021;114(June):472–89. Available from: https://doi.org/10.1016/j.tifs.2021.06.012.
63. Saini RK, Moon SH, Keum YS. An updated review on use of tomato pomace and crustacean processing waste to recover commercially vital carotenoids. Food Res Int [Internet]. 2018;108(April):516–29. Available from: https://doi.org/10.1016/j.foodres.2018.04.003.
64. Nascimento-Silva NRRD, Naves MMV. Potential of whole Pequi (Caryocar spp.) fruit-pulp, almond, oil, and Shell-as a medicinal food. J Med Food. 2019;22(9):952–62.
65. Ruelas-chacon X, Mejía-López A, Moreno-Sánchez O, Rodríguez-Guitiérrez L, Aguilera-Carbó AF, Rebolloso-Padilla ON, et al. Lycopene extraction from tomato waste assisted by cellulase and pectinase. In: Kuddus M, Aguilar C, editors. Value-addition in food products and processing through enzyme technology. Enzyme Technology; 2022. p. 283–91.
66. Chaijan M, Panpipat W. Basic composition, antioxidant activity and nanoemulsion behavior of oil from mantis shrimp (Oratosquilla nepa). Food Biosci [Internet]. 2019;31(August):100448. Available from: https://doi.org/10.1016/j.fbio.2019.100448.
67. Eismann AI, Perpetuo Reis R, Ferreira da Silva A, Negrão CD. Ulva spp. carotenoids: responses to environmental conditions. Algal Res. 2020;48(May 2019):101916.
68. Alizadeh Z, Fattahi M. Essential oil, total phenolic, flavonoids, anthocyanins, carotenoids and antioxidant activity of cultivated Damask Rose (Rosa damascena) from Iran: with chemotyping approach concerning morphology and composition. Sci Hortic (Amsterdam) [Internet]. 2021;288(May):110341. Available from: https://doi.org/10.1016/j.scienta.2021.110341.
69. Balasubramaniam V, June Chelyn L, Vimala S, Mohd Fairulnizal MN, Brownlee IA, Amin I. Carotenoid composition and antioxidant potential of Eucheuma denticulatum, Sargassum polycystum and Caulerpa lentillifera. Heliyon [Internet]. 2020;6(8):e04654. Available from: https://doi.org/10.1016/j.heliyon.2020.e04654.
70. Dulf FV, Vodnar DC, Toşa MI, Dulf EH. Simultaneous enrichment of grape pomace with γ-linolenic acid and carotenoids by solid-state fermentation with Zygomycetes fungi and antioxidant potential of the bioprocessed substrates. Food Chem. 2020;310(June 2019):125927.
71. González CM, García AL, Llorca E, Hernando I, Atienzar P, Bermejo A, et al. Carotenoids in dehydrated persimmon: antioxidant activity, structure, and photoluminescence. Lebensm Wiss Technol. 2021;142(September 2020):111007.
72. Hossain MS, Sifat SA. Din, Hossain MA, Salleh S, Hossain M, Akter S, et al. comparative assessment of bioactive compounds, antioxidant capacity and nutritional quality of red seaweeds and water spinach. Reg Stud Mar Sci [Internet]. 2021;46:101878. Available from: https://doi.org/10.1016/j.rsma.2021.101878.

73. Fernandes AS, Nascimento TC, Pinheiro PN, Vendruscolo RG, Wagner R, de Rosso VV, et al. Bioaccessibility of microalgae-based carotenoids and their association with the lipid matrix. Food Res Int. 2021;148(July):110596.
74. Jesus P d CC d, Mendes MA, Perpétuo EA, Basso TO, Nascimento CAO d. Extracellular carotenoid production and fatty acids profile of Parachlorella kessleri under increased CO2 concentrations. J Biotechnol. 2021;November 2020(329):151–9.
75. Jimenez-Escobar MP, Pascual-Mathey LI, Beristain CI, Flores-Andrade E, Jiménez M, Pascual-Pineda LA. In vitro and In vivo antioxidant properties of paprika carotenoids nano-emulsions. Lebensm Wiss Technol [Internet]. 2020;118(January 2019):108694. Available from: https://doi.org/10.1016/j.lwt.2019.108694.
76. Hu K, Peng D, Wang L, Liu H, Xie B, Sun Z. Effect of mild high hydrostatic pressure treatments on physiological and physicochemical characteristics and carotenoid biosynthesis in postharvest mango. Postharvest Biol Technol [Internet]. 2021;172(September 2020):111381. Available from: https://doi.org/10.1016/j.postharvbio.2020.111381.
77. Mendes-Silva T d CD, Vidal EE, de Souza R d FR, Schmidt K d C, Mendes PVD, da Silva Andrade RF, et al. Production of carotenoid sarcinaxanthin by Kocuria palustris isolated from Northeastern Brazil Caatinga soil and their antioxidant and photoprotective activities. Electron J Biotechnol. 2021;53:44–53.
78. Kourouma V, Mu TH, Zhang M, Sun HN. Effects of cooking process on carotenoids and antioxidant activity of orange-fleshed sweet potato. Lebensm Wiss Technol. 2019;104(January):134–41.
79. Santos JT d C, Petry FC, Tobaruela E d C, Mercadante AZ, Gloria MBA, Costa AM, et al. Brazilian native passion fruit (Passiflora tenuifila Killip) is a rich source of proanthocyanidins, carotenoids, and dietary fiber. Food Res Int. 2021;147(May):110521.
80. Vieira MV, Derner RB, Lemos-Senna E. Preparation and characterization of Haematococcus pluvialis carotenoid-loaded PLGA nanocapsules in a gel system with antioxidant properties for topical application. J Drug Deliv Sci Technol. 2021;61(September 2020):102099.
81. Ursache FM, Andronoiu DG, Ghinea IO, Barbu V, Ioniță E, Cotârleț M, et al. Valorizations of carotenoids from sea buckthorn extract by microencapsulation and formulation of value-added food products. J Food Eng. 2018;219:16–24.
82. Zeb A, Imran M. Carotenoids, pigments, phenolic composition and antioxidant activity of Oxalis corniculata leaves. Food Biosci. 2019;32(October):100472.
83. Nile A, Gansukh E, Park GS, Kim DH, Hariram NS. Novel insights on the multi-functional properties of flavonol glucosides from red onion (Allium cepa L) solid waste – in vitro and in silico approach. Food Chem. 2021;335:127650.
84. Raphaelli C d O, Azevedo JG, Pereira E d S, Vinholes JR, Camargo TM, Hoffmann JF, et al. Phenolic-rich apple extracts have photoprotective and anti-cancer effect in dermal cells. Phytomed Plus. 2021;1(4):100112.
85. Stander JH, Muller M, Joubert E, Labuschagné IF, De Beer D. Potential of low-chill requiring and pink-fleshed apple cultivars for cloudy juice production. J Food Compos Anal. 2021;103:104089.
86. Dong F, Hu J, Shi Y, Liu M, Zhang Q, Ruan J. Effects of nitrogen supply on flavonol glycoside biosynthesis and accumulation in tea leaves (Camellia sinensis). Plant Physiol Biochem. 2019;138:48–57.
87. Tomić J, Glišić I, Milošević N, Štampar F, Mikulič-Petkovšek M, Jakopič J. Determination of fruit chemical contents of two plum cultivars grafted on four rootstocks. J Food Compos Anal. 2022;105:103944.
88. Ding T, Cao K, Fang W, Zhu G, Chen C, Wang X, et al. Evaluation of phenolic components (anthocyanins, flavanols, phenolic acids, and flavonols) and their antioxidant properties of peach fruits. Sci Hortic (Amsterdam). 2020;268:109365.
89. Silva C, Câmara JS, Perestrelo R. A high-throughput analytical strategy based on QuEChERS-dSPE/HPLC–DAD–ESI-MSn to establish the phenolic profile of tropical fruits. J Food Compos Anal. 2021;98:103844.

90. Aissat AK, Chaher-Bazizi N, Richard T, Kilani-Atmani D, Pedrot E, Renouf E, et al. Analysis of individual anthocyanins, flavanols, flavonols and other polyphenols in Pistacia lentiscus L. fruits during ripening. J Food Compos Anal. 2022;106:104286.
91. Singh P, Roy TK, Kanupriya C, Tripathi PC, Kumar P, Shivashankara KS. Evaluation of bioactive constituents of Garcinia indica (kokum) as a potential source of hydroxycitric acid, anthocyanin, and phenolic compounds. Lebensm Wiss Technol. 2022;156:112999.
92. Gomes ACA, da Costa LM, de Oliveira KÁR, dos Santos LM, Magnani M, Câmara MPS, et al. Coatings with chitosan and phenolic-rich extract from acerola (Malpighia emarginata D.C.) or jabuticaba (Plinia jaboticaba (Vell.) Berg) processing by-product to control rot caused by Lasiodiplodia spp. in papaya (Carica papaya L.) fruit. Int J Food Microbiol. 2020;331:108694.
93. Liu Y, Benohoud M, Galani Yamdeu JH, Gong YY, Orfila C. Green extraction of polyphenols from citrus peel by-products and their antifungal activity against Aspergillus flavus. Food Chem X. 2021;12:100144.
94. Santarelli V, Neri L, Sacchetti G, Di Mattia CD, Mastrocola D, Pittia P. Response of organic and conventional apples to freezing and freezing pre-treatments: focus on polyphenols content and antioxidant activity. Food Chem. 2020;308:125570.
95. Ćorković I, Pichler A, Buljeta I, Šimunović J, Kopjar M. Carboxymethylcellulose hydrogels: effect of its different amount on preservation of tart cherry anthocyanins and polyphenols. Curr Plant Biol. 2021;28:100222.
96. Jaakola L, Määttä K, Pirttilä AM, Törrönen R, Kärenlampi S, Hohtola A. Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. Plant Physiol. 2002;130(2):729–39.
97. Kan J, Liu J, Xu F, Yun D, Yong H, Liu J. Development of pork and shrimp freshness monitoring labels based on starch/polyvinyl alcohol matrices and anthocyanins from 14 plants: a comparative study. Food Hydrocoll [Internet]. 2022;124(PA):107293. Available from: https://doi.org/10.1016/j.foodhyd.2021.107293.
98. Bhargav V, Kumar R, Shivashankara KS, Rao TM, Dhananjaya MV, Sane A, et al. Diversity of flavonoids profile in China aster [Callistephus chinensis (L.) Nees.] genotypes. Ind Crops Prod. 2018;111:513–9.
99. Kil YS, Choi SK, Lee YS, Jafari M, Seo EK. Chalcones from Angelica keiskei: evaluation of their heat shock protein inducing activities. J Nat Prod. 2015;78(10):2481–7.
100. Funakoshi-Tago M, Okamoto K, Izumi R, Tago K, Yanagisawa K, Narukawa Y, et al. Anti-inflammatory activity of flavonoids in Nepalese propolis is attributed to inhibition of the IL-33 signaling pathway. Int Immunopharmacol. 2015;25(1):189–98.
101. Caamal-Fuentes EE, Peraza-Sánchez SR, Torres-Tapia LW, Moo-Puc RE. Isolation and identification of cytotoxic compounds from aeschynomene fascicularis, a mayan medicinal plant. Molecules. 2015;20(8):13563–74.
102. Reynoso-Camacho R, Rodríguez-Villanueva LD, Sotelo-González AM, Ramos-Gómez M, Pérez-Ramírez IF. Citrus decoction by-product represents a rich source of carotenoid, phytosterol, extractable and non-extractable polyphenols. Food Chem. 2021;350:129239.
103. Tsiokanos E, Tsafantakis N, Termentzi A, Aligiannis N, Skaltsounis LA, Fokialakis N. Phytochemical characteristics of bergamot oranges from the Ionian islands of Greece: a multi-analytical approach with emphasis in the distribution of neohesperidose flavanones. Food Chem. 2021;343:128400.
104. de la Rosa LA, Moreno-Escamilla JO, Rodrigo-García J, Alvarez-Parrilla E. Phenolic compounds. In: Yahia E, Carrillo-Lopez A, editors. Postharvest physiology and biochemistry of fruits and vegetables. Woodhead Publishing; 2019. p. 253–71.
105. Singla RK, Dubey AK, Garg A, Sharma RK, Fiorino M, Ameen SM, et al. Natural polyphenols: chemical classification, definition of classes, subcategories, and structures. J AOAC Int. 2019;102(5):1397–400.
106. Tang Y, Xiao L, Wu X, Li W, Wu T, Zhang P. Impact of germination pretreatment on the polyphenol profile, antioxidant activities, and physicochemical properties of three color cultivars of highland barley. J Cereal Sci [Internet]. 2021;97:103152. Available from: https://doi.org/10.1016/j.jcs.2020.103152.

107. Cutrim CS, Cortez MAS. A review on polyphenols: classification, beneficial effects and their application in dairy products. Int J Dairy Technol. 2018;71(3):564–78.
108. Vardhan PV, Shukla LI. Gamma irradiation of medicinally important plants and the enhancement of secondary metabolite production. Int J Radiat Biol. 2017;93(9):967–79. https://doi.org/10.1080/09553002.2017.1344788.
109. Hong T, Benjakul S, Sae-leaw T, Khansaheb A. Trends in food science & technology protein – polyphenol conjugates: antioxidant property, functionalities and their applications. Trends Food Sci Technol. 2019;91(June):507–17.
110. Chernukha IM, Fedulova LV, Kotenkova EA. Meat by-product is a source of tissue-specific bioactive proteins and peptides against cardio-vascular diseases. Procedia Food Sci [Internet]. 2015;5:50–3. Available from: https://doi.org/10.1016/j.profoo.2015.09.013.
111. Esfandi R, Walters ME, Tsopmo A. Antioxidant properties and potential mechanisms of hydrolyzed proteins and peptides from cereals. Heliyon [Internet]. 2019;5(4):e01538. Available from: https://doi.org/10.1016/j.heliyon.2019.e01538.
112. Daroit DJ, Brandelli A. In vivo bioactivities of food protein-derived peptides – a current review. Curr Opin Food Sci. 2021;39:120–9.
113. Wen C, Zhang J, Zhang H, Duan Y, Ma H. Plant protein-derived antioxidant peptides: isolation, identification, mechanism of action and application in food systems: a review. Trends Food Sci Technol [Internet]. 2020;105(September):308–22. Available from: https://doi.org/10.1016/j.tifs.2020.09.019.
114. El-Saadony MT, S. F. Khalil O, Osman A, Alshilawi MS, Taha AE, Aboelenin SM, et al. Bioactive peptides supplemented raw buffalo milk: biological activity, shelf life and quality properties during cold preservation. Saudi. J Biol Sci. 2021;28(8):4581–91.
115. Przybylski R, Firdaous L, Châtaigné G, Dhulster P, Nedjar N. Production of an antimicrobial peptide derived from slaughterhouse by-product and its potential application on meat as preservative. Food Chem [Internet]. 2016;211:306–13. Available from: https://doi.org/10.1016/j.foodchem.2016.05.074.
116. Lorenzo JM, Munekata PES, Gómez B, Barba FJ, Mora L, Pérez-Santaescolástica C, et al. Bioactive peptides as natural antioxidants in food products – a review. Trends Food Sci Technol [Internet]. 2018;79(July):136–47. Available from: https://doi.org/10.1016/j.tifs.2018.07.003.
117. Zhan Q, Wang Q, Liu Q, Guo Y, Gong F, Hao L, et al. The antioxidant activity of protein fractions from Sacha inchi seeds after a simulated gastrointestinal digestion. Lebensm Wiss Technol [Internet]. 2021;145(November 2020):111356. Available from: https://doi.org/10.1016/j.lwt.2021.111356.
118. Pan C, Ma J, Tao F, Ji C, Zhao Y, Chen S, et al. Novel insight into the antioxidant proteins derived from laver (Porphyra haitanensis) by proteomics analysis and protein based bioinformatics. Food Biosci [Internet]. 2021;42(April):101134. Available from: https://doi.org/10.1016/j.fbio.2021.101134.
119. Di Bernardini R, Harnedy P, Bolton D, Kerry J, O'Neill E, Mullen AM, et al. Antioxidant and antimicrobial peptidic hydrolysates from muscle protein sources and by-products. Food Chem. 2011;124(4):1296–307.
120. Zhao Y, Wang C, Lu W, Sun C, Zhu X, Fang Y. Evolution of physicochemical and antioxidant properties of whey protein isolate during fibrillization process. Food Chem [Internet]. 2021;357(April):129751. Available from: https://doi.org/10.1016/j.foodchem.2021.129751.
121. Arihara K, Yokoyama I, Ohata M. Bioactivities generated from meat proteins by enzymatic hydrolysis and the Maillard reaction. Meat Sci. 2021;180(May):108561.
122. Sila A, Bougatef A. Antioxidant peptides from marine by-products: isolation, identification and application in food systems. J Funct Foods [Internet]. 2016;21:10–26. Available from: https://doi.org/10.1016/j.jff.2015.11.007.
123. Cui Q, Sun Y, Cheng J, Guo M. Effect of two-step enzymatic hydrolysis on the antioxidant properties and proteomics of hydrolysates of milk protein concentrate. Food Chem [Internet]. 2022;366(July 2021):130711. Available from: https://doi.org/10.1016/j.foodchem.2021.130711.

124. Zhang D, Chen L, Cai J, Dong Q, Din Z u, Hu ZZ, et al. Starch/tea polyphenols nanofibrous films for food packaging application: from facile construction to enhance mechanical, antioxidant and hydrophobic properties. Food Chem [Internet]. 2021;360(December 2020):129922. Available from: https://doi.org/10.1016/j.foodchem.2021.129922.
125. Tong X, Cao J, Tian T, Lyu B, Miao L, Lian Z, et al. Changes in structure, rheological property and antioxidant activity of soy protein isolate fibrils by ultrasound pretreatment and EGCG. Food Hydrocoll [Internet]. 2022;122(August 2021):107084. Available from: https://doi.org/10.1016/j.foodhyd.2021.107084.
126. Daliri H, Ahmadi R, Pezeshki A, Hamishehkar H, Mohammadi M, Beyrami H, et al. Quinoa bioactive protein hydrolysate produced by pancreatin enzyme- functional and antioxidant properties. Lebensm Wiss Technol [Internet]. 2021;150(June):111853. Available from: https://doi.org/10.1016/j.lwt.2021.111853.
127. Gao J, Li T, Chen D, Gu H, Mao X. Identification and molecular docking of antioxidant peptides from hemp seed protein hydrolysates. Lebensm Wiss Technol [Internet]. 2021;147(April):111453. Available from: https://doi.org/10.1016/j.lwt.2021.111453.
128. Gómez A, Gay C, Tironi V, Avanza MV. Structural and antioxidant properties of cowpea protein hydrolysates. Food Biosci. 2021;41(January):101074.
129. Habinshuti I, Mu TH, Zhang M. Structural, antioxidant, aroma, and sensory characteristics of Maillard reaction products from sweet potato protein hydrolysates as influenced by different ultrasound-assisted enzymatic treatments. Food Chem [Internet]. 2021;361(April):130090. Available from: https://doi.org/10.1016/j.foodchem.2021.130090.
130. Liu C, Hu B, Cheng Y, Guo Y, Yao W, Qian H. Carotenoids from fungi and microalgae: a review on their recent production, extraction, and developments. Bioresour Technol [Internet]. 2021;337(June):125398. Available from: https://doi.org/10.1016/j.biortech.2021.125398.
131. Ryder K, Bekhit AE, Mcconnell M, Carne A. Towards generation of bioactive peptides from meat industry waste proteins: generation of peptides using commercial microbial proteases. Food Chem [Internet]. 2016;208:42–50. Available from: https://doi.org/10.1016/j.foodchem.2016.03.121.
132. Joseph-Leenose-Helen J, Noor N, Mushtaq M, Gani A. Ultrasonics as a tool for development of pine-needle extract loaded bee wax edible packaging for value addition of Himalayan cheese. Ultrason Sonochem. 2022;82:105914.
133. Choi I, Li N, Zhong Q. Co-loading curcumin and quercetin in freeze-dried mushroom microparticles to inhibit lipid oxidation in beef patties. Food Chem. 2022;374:131625.
134. Mahmoudi L, Tavakoilpour H, Roozbeh-Nasiraie L, Kalbasi-Ashtari A. Ultrasonication and encapsulation of Butcher broom (Ruscus Hyrcanus L.) extract and its bioactive effects on qualitative properties, oxidative stability and shelf life of cake. Sustain Chem Pharm. 2020;17:100295.
135. Deseta ML, Sponton OE, Erben M, Osella CA, Frisón LN, Fenoglio C, et al. Nanocomplexes based on egg white protein nanoparticles and bioactive compounds as antifungal edible coatings to extend bread shelf life. Food Res Int. 2021;148:110597.
136. Ma Y, Zhao H, Ma Q, Cheng D, Zhang Y, Wang W, et al. Development of chitosan/potato peel polyphenols nanoparticles driven extended-release antioxidant films based on potato starch. Food Packag Shelf Life. 2022;31:100793.
137. Esmaeili H, Cheraghi N, Khanjari A, Rezaeigolestani M, Basti AA, Kamkar A, et al. Incorporation of nanoencapsulated garlic essential oil into edible films: a novel approach for extending shelf life of vacuum-packed sausages. Meat Sci. 2020;166:108135.
138. Flakelar CL, Prenzler PD, Luckett DJ, Howitt JA, Doran G. A rapid method for the simultaneous quantification of the major tocopherols, carotenoids, free and esterified sterols in canola (Brassica napus) oil using normal phase liquid chromatography. Food Chem. 2017;214:147–55.
139. Martins FCOL, Sentanin MA, De Souza D. Analytical methods in food additives determination: compounds with functional applications. Food Chem [Internet]. 2019;272(April 2018):732–50. Available from: https://doi.org/10.1016/j.foodchem.2018.08.060.

140. Padhi EMT, Liu R, Hernandez M, Tsao R, Ramdath DD. Total polyphenol content, carotenoid, tocopherol and fatty acid composition of commonly consumed Canadian pulses and their contribution to antioxidant activity. J Funct Foods. 2017;38:602–11.
141. Neri-Numa IA, Arruda HS, Geraldi MV, Maróstica Júnior MR, Pastore GM. Natural prebiotic carbohydrates, carotenoids and flavonoids as ingredients in food systems. Curr Opin Food Sci. 2020;33:98–107.
142. Pinheiro Bruni G, de Oliveira JP, Gómez-Mascaraque LG, Fabra MJ, Guimarães Martins V, Zavareze E d R, et al. Electrospun β-carotene–loaded SPI:PVA fiber mats produced by emulsion-electrospinning as bioactive coatings for food packaging. Food Packag Shelf Life [Internet]. 2020;23(October 2019):100426. Available from: https://doi.org/10.1016/j.fpsl.2019.100426.
143. Martins AJ, Cerqueira MA, Cunha RL, Vicente AA. Fortified beeswax oleogels: effect of β-carotene on the gel structure and oxidative stability. Food Funct. 2017;8(11):4241–50.
144. Stoll L, Rech R, Flôres SH, Nachtigall SMB, de Oliveira RA. Poly(acid lactic) films with carotenoids extracts: release study and effect on sunflower oil preservation. Food Chem. 2019;281(December 2018):213–21.
145. Kadam AA, Singh S, Gaikwad KK. Chitosan based antioxidant films incorporated with pine needles (Cedrus deodara) extract for active food packaging applications. Food Control. 2021;124:107877.
146. Zhang L, Zhang Z, Chen Y, Ma X, Xia M. Chitosan and procyanidin composite films with high antioxidant activity and pH responsivity for cheese packaging. Food Chem. 2021;338:128013.
147. El-Sayed SM, El-Sayed HS, Ibrahim OA, Youssef AM. Rational design of chitosan/guar gum/zinc oxide bionanocomposites based on Roselle calyx extract for Ras cheese coating. Carbohydr Polym. 2020;239:116234.
148. Hosseini SF, Ghaderi J, Gómez-Guillén MC. Tailoring physico-mechanical and antimicrobial/antioxidant properties of biopolymeric films by cinnamaldehyde-loaded chitosan nanoparticles and their application in packaging of fresh rainbow trout fillets. Food Hydrocoll. 2022;124:107249.
149. Lim JY, Lee CL, Kim GH, Bang YJ, Rhim JW, Yoon KS. Using lactic acid bacteria and packaging with grapefruit seed extract for controlling Listeria monocytogenes growth in fresh soft cheese. J Dairy Sci. 2020;103(10):8761–70.
150. Alirezalu K, Hesari J, Nemati Z, Munekata PES, Barba FJ, Lorenzo JM. Combined effect of natural antioxidants and antimicrobial compounds during refrigerated storage of nitrite-free frankfurter-type sausage. Food Res Int. 2019;120:839–50.
151. Sani IK, Geshlaghi SP, Pirsa S, Asdagh A. Composite film based on potato starch/apple peel pectin/ZrO2 nanoparticles/ microencapsulated Zataria multiflora essential oil; investigation of physicochemical properties and use in quail meat packaging. Food Hydrocoll. 2021 Aug;117:106719.
152. Rostamabadi H, Falsafi SR, Jafari SM. Nanoencapsulation of carotenoids within lipid-based nanocarriers. J Control Release [Internet]. 2019;298(February):38–67. Available from: https://doi.org/10.1016/j.jconrel.2019.02.005.
153. Fernandes R d PP, Trindade MA, de Melo MP. Natural antioxidants and food applications: healthy perspectives. Altern Replace Foods. 2018;17:31–64.
154. Tkaczewska J. Peptides and protein hydrolysates as food preservatives and bioactive components of edible films and coatings – a review. Trends Food Sci Technol [Internet]. 2020;106:298–311. Available from: https://doi.org/10.1016/j.tifs.2020.10.022.
155. Kęska P, Stadnik J. Characteristic of antioxidant activity of dry-cured pork loins inoculated with probiotic strains of LAB probiotic strains of LAB. CyTA J Food. 2017;15(3):374–81.

Chapter 3
Natural Antimicrobials

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3.1 Introduction

Over the last decades, health problems have generated great concern. The first one is the appearance of several foodborne diseases caused by different agents such as bacteria, viruses, and fungi. The second problem involves the indiscriminate use of antibiotics, which have led to the appearance of resistant bacteria [1]. Likewise, the food market has an increasing demand for "green" products that contain the least amount of synthetic molecules. The main alternative for this problem is to replace synthetic with natural antimicrobials isolated from vegetal, microbial, and animal origins, such as essential oils, bacteriocins, polyphenols and enzymes, among others [2]. These molecules have different mechanisms of action against bacteria, reducing the possibility of generating resistance, and furthermore, several of them, especially the plant based have potential health benefits for humans such as antioxidant and anticancer activity. This chapter aimed to review the main natural antimicrobials and their food applications.

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3.2 Main Natural Antimicrobials

3.2.1 Essential Oils

Essential oils (EOs) are among the most promising natural antimicrobials for the food, cosmetic and pharmaceutical industries. They can be defined as volatile substances naturally produced in different parts of the plants such as buds, stems, leaves, flowers, twigs, roots, seeds, and wood [3]. Although called oils, EOs are not strictly oils as they tend to have low water solubility. Furthermore, they tend to have an odor and sometimes a distinctive taste, a characteristic that is used by plants against herbivores [4]. Furthermore, EOs are complex mixtures of often hundreds of

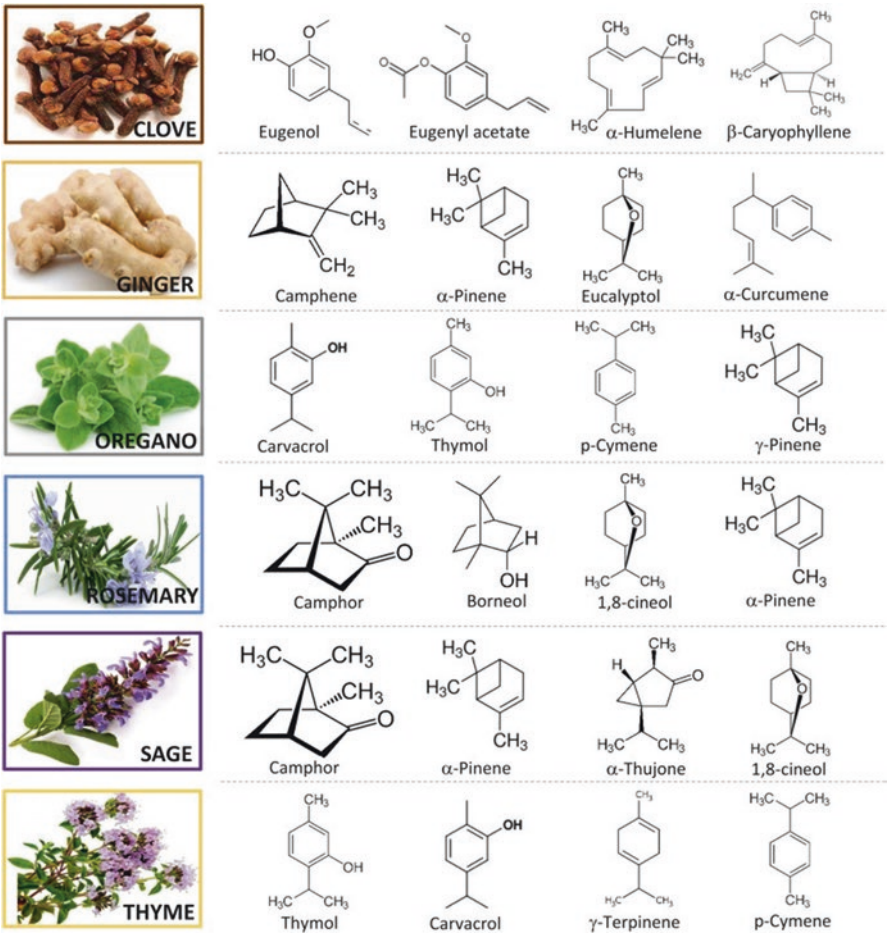

Fig. 3.1 Main components of essential oils extracted from clove, ginger, oregano, rosemary, sage, and thyme. (Adapted with permission [6])

volatile molecules, and their composition is heavily dependent on the plant's growth condition [5]. Figure 3.1 shows the main components of EOs extracted from different plants. The antimicrobial, antifungal, and antiviral activities of EOs are well known and are highly dependent on their composition. It has been reported that EOs containing aldehydes or phenols as major components present the highest antimicrobial activity followed by EOs in which terpene alcohols are the main components. Furthermore, EOs containing ketone or esters have a weaker antimicrobial activity, while EOs containing terpene hydrocarbons are usually inactive [6–8].

Over the years, the activity of EOs against food borne pathogens have been tested and results have shown that their antimicrobial mechanism is heavily dependent on their composition [9]. The antimicrobial activity of EOs is determined by their main components or synergistic effects of various components, likewise, the action mechanism of every major component could be different, leading to several pathways to bacterial killing. The antimicrobial mechanisms of EOs include effects on the bacterial cell wall, disruptions of the cell membrane, effects on the RNA and DNA that break the normal reproduction of the genetic material of the bacteria, furthermore, they can interfere with the absorption, transport, and metabolism of nutrients by the bacteria [9–11]. Figure 3.2 shows a schematic representation of several inhibition forms of EOs in pathogenic bacteria.

The complex nature of EOs and their wide range of antimicrobial mechanisms allows them to act against a variety of microorganisms. For instance, *Rosmarinus officinalis* EO has shown activity against both gram-positive (*Lactococcus lactis,*

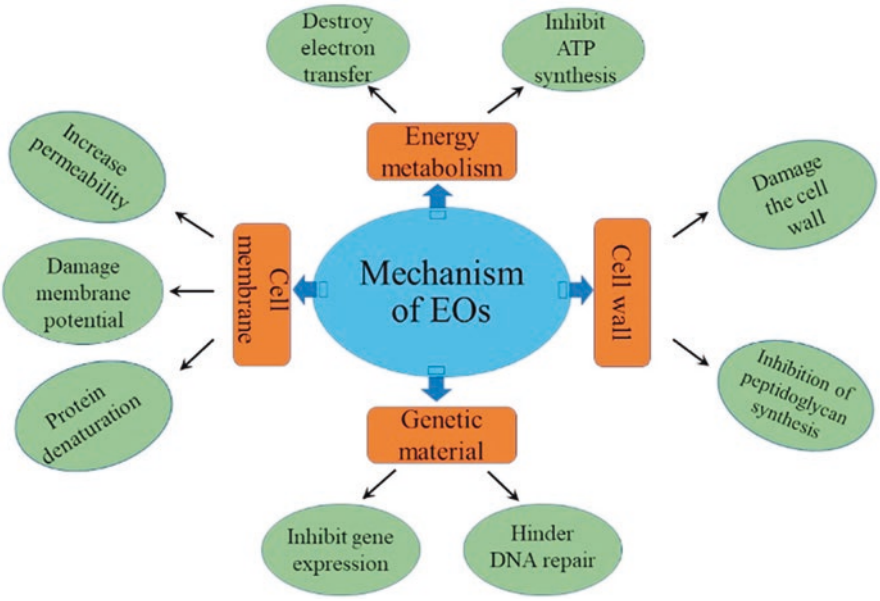

Fig. 3.2 Different types of mechanism of action of EOs against bacterial cells. (Adapted with permission [12])

S. aureus, L. monocytogenes, Brochotrix thermosphacta, Leuconostoc mesenteroides, Micrococcus luteus, and Lactobacillus casei) and gram-negative (*P. aeruginosa, E. coli, Shigella dysanteriae, S. typhimurium, Salmonella enteritidis, and Yersinia enterocolitica*) bacteria. Likewise, EO from *Thymus vulgaris* is known for its activity against the gram-positive *S. aureus, Streptococcus pyogenes* and the gram-negative *P. aeruginosa, E. coli* and *S. typhimurium* [13, 14]. One of the most promising EOs for antimicrobial applications has been isolated from the *Lippia* genus, which has shown activity against some of the most common foodborne bacteria and fungus [15–17]. Furthermore, EOs from *Zingiber officinale, Salvia Rosmarinus, Citrus sinensis, Origanum vulgare, Cymbopogon flexuosus* have shown activity against several types of bacteria, as shown in Table 3.1.

3.2.2 Polyphenols

Polyphenols are among the most common secondary metabolites of plants. They are synthetized by plants as a defense against herbivores, pathogens, and different stress conditions [27]. Polyphenols comprise a wide group of molecules characterized by at least two phenyl rings and one or more hydroxyl substituents. Polyphenols can be

Table 3.1 Some recent studies related to the antimicrobial activity of several essential oils

Essential oil	Microorganism	Reference
Thymus vulgaris	*E. Coli*	[18–20]
	P. aeruginosa	
	B. cereus	
	Salmonella enterica	
Rosmarinus officinalis	*E. Coli*	[21, 22]
	S. Aureus	
Zingiber officinale	*S. Aureus*	[23]
	B. subtilis	
	E. Coli	
	P. aeruginosa	
Salvia Rosmarinus	*E. Coli*	[24]
	S. Aureus	
	Proteus mirabilis	
Origanum vulgare	*E. Coli*	[25]
	S. Aureus	
	P. aeruginosa	
	B. subtilis	
Cymbopogon flexuosus	*C. grubii*	[26]
	S. Aureus	
	P. aeruginosa	

classified by their botanical origin and biological function, however, the main classification comes from their chemical structure, as they can be divided into flavonoids and non-flavonoids polyphenols [28, 29]. The first group is the largest and includes many subcategories such as flavanols, anthocyanidins, anthocyanins, isoflavones, flavones, flavonols, flavanones, and flavanonols. The second group includes molecules such as curcumin and caffeic acid, among others [28, 29].

3.2.3 Flavonoids

Although flavonoids are the largest family of secondary metabolites in nature, and almost nine thousand flavonoid molecules have been identified, they share a basic chemical structure [30]. Flavonoids contain three phenolic rings, namely A (6 carbon) and B (6-carbon) linked with the central C (3-carbon) ring; C6-C3-C6 [31]. This type of structure allows the formation of several derivatives compounds, with distinct substitutions in the basic structure. The basic structure of some of the most common sub-groups of flavonoids is shown in Fig. 3.3.

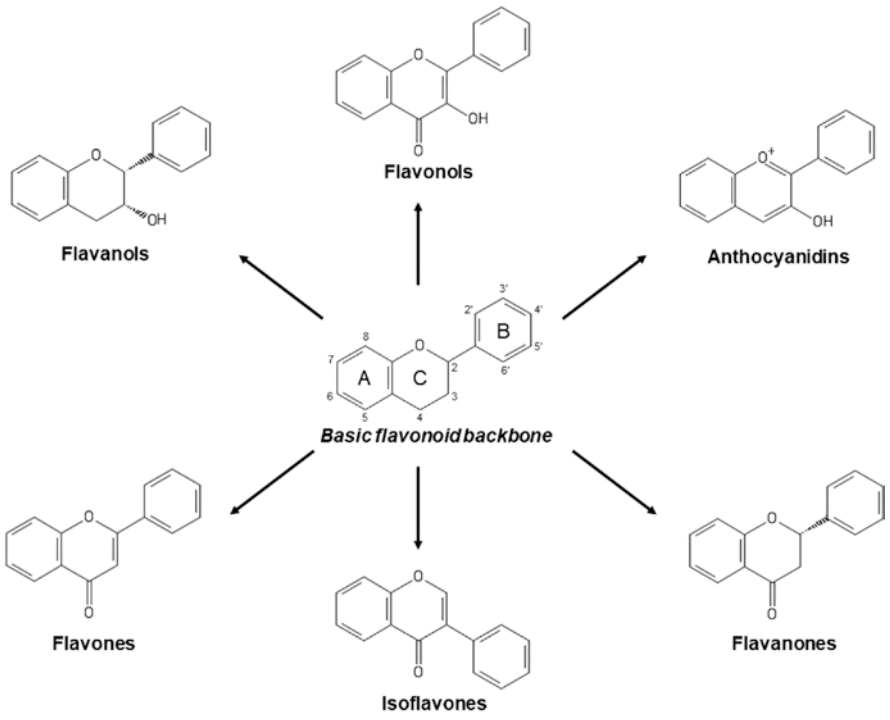

Fig. 3.3 Basic structure of the several flavonoids subgroups. (Adapted with permission [33])

Due to the heterogeneous structure of flavonoids, there is not a single antimicrobial mechanism related to them, and some of them can act through several inhibition pathways [32]. Some flavonoids disrupt the cell membrane function leading to bacterial death. In the literature has been reported that the antimicrobial activity of catechins involves the partition of the more non-polar compounds in the hydrophobic interior of the membrane, and the formation of hydrogen bonds between the polar head groups of the membrane lipids and the more hydrophilic flavonoids, leading to structural damages in the cell membrane [33, 34]. Furthermore, catechins contribute to the bacterial membrane rupture by inactivating or inhibiting the synthesis of intracellular and extracellular enzymes [35].

Other inhibition mechanisms include the inhibition of the fatty acid synthase-type II (FAS- II) pathway, leading to a stop in bacterial cell envelop synthesis. Furthermore, flavonoids have shown to have an inhibitory effect on bacterial topoisomerase, stopping nucleic acid synthesis. Finally, some flavonoids affect the electron transfer chain [36, 37]. Due to their heterogeneous structure and inhibition mechanism, several flavonoids can act on different types of bacteria. Table 3.2 shows some of the most recent studies of flavonoids and their antimicrobial activity.

Table 3.2 Studies related to the antimicrobial activity of several flavonoids

Flavonoid	Microorganism	Reference
Naringeni	*S. aureus*	[38–40]
	P. aeruginosa	
	E. coli	
	Listeria monocytogenes	
Quercetin	*P. aeruginosa*	[41, 42]
	H. pylori	
	S. aureus	
	Listeria monocytogenes	
Baicalin	*S. aureus*	[43–45]
	H. pylori	
	E. Coli	
Chatechin	*Salmonella enterica*	[46, 47]
	E. Coli	
Anthocyanins (from Vaccinium corymbosum)	*P. Aeruginosa*	[48]
	E. coli	
	Proteus mirabilis	
	Acinetobacter baumannii	
	S. aureus	
Anthocyanins (from Maloideae subfamily)	*E. coli*	[49]
	Morganella morganii	
	P. aeruginosa	
	E. faecalis	
	E. faecium	
	S. aureus	

3.2.4 Non-Flavonoids Polyphenols

One of the most studied non-flavonoid polyphenols is curcumin, a highly hydrophobic, yellow polyphenol extracted from turmeric (*Curcuma longa)* [50]. Curcumin has shown a broad-spectrum of antibacterial activity [51, 52]. The mechanism of the antimicrobial activity of curcumin takes place through the interaction with the essential cell division initiating protein FtsZ [53]. Likewise, curcumin has shown the capability to inhibit bacterial growth by targeting the bacterial cell membrane, cell wall and other cellular structures [52].

As with other natural antimicrobials, curcumin presents antimicrobial activity and it is heavily dependent on the bacteria type and strain [54]. In the literature have been shown the efficiency of curcumin against gram-positive bacteria, especially *S. aureus, S. epidermidis, Streptococcus pyogenes, M. luteus* [51, 55, 56]. Likewise, some reports of its antimicrobial activity against Gram-negative bacteria such as *E. coli* and *Pseudomonas aeruginosa* [57, 58].

Another non-flavonoid of great interest is resveratrol, a molecule that is found in the roots of white hellebore, grapes, berries, red wine, and nuts [59]. Resveratrol is a versatile molecule that interacts with more than 20 proteins in eukaryotic organisms leading to several pathways to antimicrobial inhibition. These include binding to ATP synthase, thus, partially inhibiting both ATP hydrolysis and ATP synthesis functions of the ATP synthase [60]. Furthermore, resveratrol induces DNA fragmentation and inhibition of the FtsZ-mediated septum formation. Finally, resveratrol can lead to membrane damage of cells [61, 62]. Bacteria that have shown susceptibility to resveratrol include *B. cereus*, *M. smegmatis*, *H. pylori*, *S. aureus* and *E. coli,* among others [63–65].

Caffeic acid is another non-flavonoid polyphenol that is found in several fruits, wine, coffee, olive oil, and legumes. It can be found in different forms such as monomeric, dimeric, trimeric, and oligomeric derivatives of sugar esters, organic esters, glycosides, and amides [66]. Studies have shown that caffeic acid inhibits the growth of *S. aureus* [67, 68], *E. Coli* [69], *S. enterica, S. epidermidis, Proteus mirabilis* [70], among others.

3.2.5 Bacteriocins

Bacteriocins are a heterogeneous group of bioactive material produced by bacteria that includes peptides and proteins that have shown antimicrobial activity against other bacteria [71]. Bacteriocins peptides are ribosomally synthesized and secreted by a variety of bacteria for the purpose of killing other bacteria, thus removing the microbial competition in prokaryotes [72]. The structure, physicochemical and biochemical properties of bacteriocins vary widely, as well as their antimicrobial activity. They could either manifest antimicrobial activity directed against the same bacterial strains that produced them or against strains of closely related species [72].

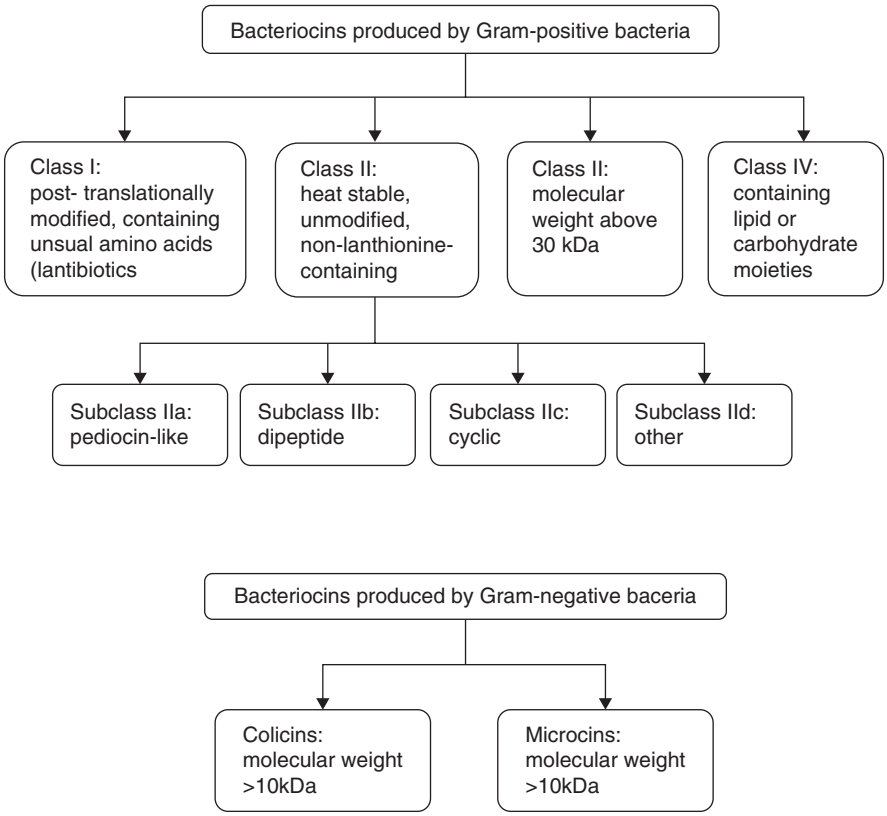

Fig. 3.4 Classification of bacteriocins produced by gram positive and gram-negative bacteria. (Adapted with permission [71])

Bacteriocins can be produced by both gram-positive and gram-negative bacteria and there are some reports of bacteriocins produced by archea [73]. Although both bacteriocins produced by gram-positive and gram-negative bacteria form and heterogeneous groups of molecules, they can be organized into different subclasses that have similar properties, as shown in Fig. 3.4.

Class I from the bacteriocins produced by gram-positive bacteria include lantibiotics of molecular weight below 5 kDa and contain atypical amino acids, such as lanthionine, methyllanthionine, dehydroalanine, dehydrobutyrine, and D-alanine. Among this class is nisin a bacteriocin produced by certain strains of *Lactococcus lactis*. Nisin has shown antimicrobial activity against gram-positive bacteria from the genera *Lactococcus, Lactobacillus, Streptococcus, Staphylococcus, Micrococcus, Pediococcus, Listeria, Bacillus* and *Clostridium* [74]. Class II includes non-lantibiotic bacteriocins with a molecular weight below 10 kDa that do not contain lanthionine in their composition [75]. Among this subclass, the most promising bacteriocins are sakacins and pediocins. The first one is produced by certain strains

of Lb. sakei strains and that is known for its antimicrobial activity against some strains of *Lactobacillus spp*. and *Listeria spp* [75]. Pediocins are produced by several strains of *Pediococcus acidilactici*, *P. pentosaceus* and *Lactobacillus plantarum.* Pediocins have shown activity against *L. monocytogenes* and *L.innocua* [76].

Class III from the bacteriocins produced by gram-positive bacteria includes molecules of molecular weight above 30 kDa and that are thermolabile. This class is generally sub-divided into two groups; the first group is composed of the bacteriolysins such as the lysostaphin produced by *S. aureus* and enterolysin A produced by Enterococcus faecalis and nonlytic antimicrobial proteins such as helveticin J produced by *Lactobacillus helveticus* [77]. Finally, Class IV includes bacteriocins, which are more complex systems that require lipid or carbohydrate moieties in their molecule.

Bacteriocins produced by gram-negative bacteria can be divided in two groups: Colicins and microcins. They are synthetized by most of *E. coli* strains and some bacteria from the *Klebsiella* strains [71]. They have been less studied than their gram-positive produce counterpart and have a narrower antimicrobial activity.

3.2.6 Natamycin

Natamycin is a tetraene polyene macrolide that is produced by fermentation of the bacterium *Streptomyces natalensis* and closely related species [78, 79]. One of the main characteristics of natamycin is that it has high antifungal activity against nearly all yeasts and moulds but has no effect on bacteria, protozoa or viruses [80]. Natamycin is known to target ergosterol, in the cell membrane and since it is absent from the cell membranes of viruses and bacteria these microorganisms are resistant to this molecule. In general, the antifungal activity of natamycin can be attributed to the inhibition of ergosterol-dependent transport of glucose and amino acid transport across the cell membrane [81–83].

Natamycin has shown antifungal activity against *Brettanomyces bruxellensis, C. albicans, C. krusei H66, C. pseudotropicalis H3, C. valida H74, C. vini, Debaryomyces hansenii H42, Dekkera bruxellensis (Strains CBS2796, CBS4459, CBS6055), Hanseniasporum uvarum CBS5074, Hansenula polymorpha, Pichia membranaefaciens H67, Rhodotorula mucilaginosa CBS816* and *Aspergillus niger*, among others [83].

3.2.7 Reuterin

Reuterin (β-hydroxypropionaldehyde) is an organic molecule produced by some strains of *Lactobacillus reuteri* during anaerobic fermentation of glycerol [84]. Reuterin has shown inhibitory action against several gram-positive and gram-negative bacteria, spores, fungi, and protozoa [85]. Reuterin induces oxidative stress

in the bacterial cells by interacting with thiol groups [86], this molecule has shown activity against bacteria such as: *E. coli, P. aeruginosa*, *S. aureus*, *B. subtilis* and *L. monocytogenes* [87, 88].

3.2.8 Lysozyme

Lysozyme is an antimicrobial enzyme, also called muramidase or N-acetylmuramic hydrolase. Its main action is to hydrolyze the β-1,4-linkages between N-acetyl-d-glucosamine and N-acetylmuramic acid residues in the peptidoglycan of bacterial cell walls, damaging the cell membrane [89]. Lysozyme has shown antimicrobial activity against gram-positive bacteria such as *L. monocytogenes, C. botulinum, T. thermosaccharolyicum, G. stearothermophilus, S. aureus, Bacillus stearothermophilus, and Bacillus coagulans* [90]. Lysozyme is found as an additive in several food products in order to prevent the growth of the bacteria on food materials, this enables long-term storage for plenty of vegetables, milk, fish, and meat. Although lysozyme can be found in several mammals, the major natural source of this enzyme is chicken egg white, however, only about 3.5% of egg white is lysozyme providing a limitation to further industrial uses [91, 92].

3.2.9 Lactoperoxidase and Lactoferrin

Lactoperoxidase is an enzyme found in milk that contains both a heme group and a single glycoprotein chain of around 608 amino acids. Its molecular weight is ~78 kDa [93, 94]. In order to add an antimicrobial activity, lactoperoxidase needs to be accompanied by the thiocyanate ion (SCN^-) and hydrogen peroxide (H_2O_2), commonly called as lactoperoxidase system or LPO system.

The LPO system has shown great antimicrobial activity, which is the result of the oxidation of –SH groups by $OSCN^-/O_2SCN^-$ in enzymes, such as hexokinase and glyceraldehydes-3-phosphate dehydrogenase. Likewise, the inhibitory effect of the LPO system may vary from oxidative killing to blockage of the sugar transport system or interference with cytopathic effects [95, 96]. Both gram-positive and gram-negative bacteria have been shown to be affected by the LPO system; as shown by the inhibition of *Salmonella typhimurium, L. monocytogenes, Bacillus cereus b, S. Aureus,* and *E. Coli* [97].

Lactoferrin is another protein found in milk with potential applications as a natural antimicrobial in food conservation. This iron binding glycoprotein is a single-chain protein that contains 703 amino acids folded into two globular lobes. Lactoferrin is present in milk and colostrum, being strongly involved in immunological processes response to pathogens, as well as inflammatory disorders, including allergy, arthritis, and cancer [98]. This glycoprotein has shown antimicrobial activity against bacteria that need ferric ions for their growth, sequestering the iron

atoms from bacterial pathogens, thus inhibiting bacterial growth. This includes bacteria such as *E. coli, Salmonella typhi, Shigella flexneri, Shigella dysenteriae, Aeromonas hydrophila, S. aureus,* and *L. monocytogenes* [99].

3.3 Application of Natural Antimicrobial in Food Preservation

In general, the use of natural antimicrobials for extending shelf life can be divided into two main approaches. The first one has been the most common and is based on the inclusion of the antimicrobial in the food matrix, while the second one is the development of active food packaging that releases the natural antimicrobials into the food product during storage. The second approach has been greatly studied in the last decades as it does not affect the food product composition and sensory properties.

Currently, active food packaging based on natural polymers and EOs has generated great interest with several studies reporting their capability to extend shelf life of food products. The incorporation of effects the microstructure of the packaging material, increasing the tensile strength and elongation at break, decreasing water vapor permeability, and changing color and transparency [12, 100]. Although changing the properties of the packaging material is significant, the most important factor for the inclusion of EOs is the migration of bioactive molecules that enhance the antibacterial and antioxidant properties of packaging materials. Due to the small size of most of the components of the EOs they tend to migrate from the packaging migrate to the food too quickly, and not in the controlled manner that is expected in an active packaging [12]. To overcome this, the use of nanocarrires, such as polymeric nanoparticles, nanoclays and nanoemulsions has controlled the release of the bioactive molecules into the food product. This same approach has been used for the inclusion of other natural antimicrobials in active food packaging. Some examples include the use of polyphenols such as curcumin. The antimicrobial and antifungal activity of films made from chitosan and curcumin was measure against common food pathogenic agents: *S. Aureus* and *Rhizoctonia solani*, Results showed that curcumin slightly increased the antimicrobial activity of the chitosan films against both microorganism [101]. Further studies have shown curcumin-chitosan films have antimicrobial activity against *Salmonella* and *E. Coli* [102, 103].

3.4 Conclusions

Natural antimicrobials from different sources (plants, animals, and microorganisms) have become one of the main alternatives to the synthetic antimicrobials normally used in the food industry. They contain all the qualities to be used as

preservatives with a wide range of bacteria and fungi affected by them. However, the effective use of these molecules in the food industry still requires further studies on their toxicity and long term effects on the consumers. Likewise, it is necessary to increase knowledge on how they behave in normal manufacturing conditions (thermal processing, freezing, among others) in order to fully implement them.

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References

1. Kirbis A, Krizman M. Spread of antibiotic resistant bacteria from food of animal origin to humans and vice versa. Procedia Food Sci. 2015;5:148–51.
2. Bahrami A, Delshadi R, Assadpour E, Jafari SM, Williams L. Antimicrobial-loaded nanocarriers for food packaging applications. Adv Colloid Interf Sci. 2020;278:102140.
3. Tariq S, Wani S, Rasool W, Shafi K, Bhat MA, Prabhakar A, et al. A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. Microb Pathog. 2019;134:103580.
4. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – a review. Food Chem Toxicol. 2008;46(2):446–75.
5. Teles S, Pereira JA, Santos CHB, Menezes RV, Malheiro R, Lucchese AM, et al. Effect of geographical origin on the essential oil content and composition of fresh and dried Mentha×villosa Hudson leaves. Ind Crop Prod. 2013;46:1–7.
6. Pateiro M, Barba FJ, Domínguez R, Sant'Ana AS, Mousavi Khaneghah A, Gavahian M, et al. Essential oils as natural additives to prevent oxidation reactions in meat and meat products: a review. Food Res Int. 2018;113:156–66.
7. Inouye S, Yamaguchi H, Takizawa T. Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. J Infect Chemother. 2001;7(4):251–4.
8. Delaquis PJ, Stanich K, Girard B, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int J Food Microbiol. 2002;74(1):101–9.
9. Ju J, Xie Y, Guo Y, Cheng Y, Qian H, Yao W. The inhibitory effect of plant essential oils on foodborne pathogenic bacteria in food. Crit Rev Food Sci Nutr. 2019;59(20):3281–92.
10. Álvarez-Martínez FJ, Barrajón-Catalán E, Herranz-López M, Micol V. Antibacterial plant compounds, extracts and essential oils: an updated review on their effects and putative mechanisms of action. Phytomedicine. 2021;90:153626.
11. Rao J, Chen B, McClements DJ. Improving the efficacy of essential oils as antimicrobials in foods: mechanisms of action. Annu Rev Food Sci Technol. 2019;10(1):365–87.
12. Ni Z-J, Wang X, Shen Y, Thakur K, Han J, Zhang J-G, et al. Recent updates on the chemistry, bioactivities, mode of action, and industrial applications of plant essential oils. Trends Food Sci Technol. 2021;110:78–89.
13. Micucci M, Protti M, Aldini R, Frosini M, Corazza I, Marzetti C, et al. Thymus vulgaris L. essential oil solid formulation: chemical profile and spasmolytic and antimicrobial effects. Biomolecules. 2020;10(6):860.

14. Rota MC, Herrera A, Martínez RM, Sotomayor JA, Jordán MJ. Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis and Thymus hyemalis essential oils. Food Control. 2008;19(7):681–7.
15. de Almeida WS, de Lima SG, Barreto HM, Andrade LMS, Fonseca L, Athayde Sobrinho C, et al. Chemical composition and antimicrobial activity of the essential oil of Lippia lasiocalycina Cham. (Verbenaceae). Ind Crop Prod. 2018;125:236–40.
16. Ribeiro FP, Santana de Oliveira M, de Oliveira Feitosa A, Santana Barbosa Marinho P, Moacir do Rosario Marinho A, de Aguiar Andrade EH, et al. Chemical composition and antibacterial activity of the Lippia origanoides kunth essential oil from the Carajás National Forest, Brazil. Evid Based Complement Alternat Med. 2021;2021:9930336.
17. Hernandes C, Pina ES, Taleb-Contini SH, Bertoni BW, Cestari IM, Espanha LG, et al. Lippia origanoides essential oil: an efficient and safe alternative to preserve food, cosmetic and pharmaceutical products. J Appl Microbiol. 2017;122(4):900–10.
18. Almasi L, Radi M, Amiri S. The release rate and antimicrobial activity of calcium-alginate films containing self-microemulsifying Thymus vulgaris essential oil against Escherichia coli and Staphylococcus aureus. J Food Saf. 2020;40(5):e12828.
19. Gonçalves ND, FdL P, Sartoratto A, Derlamelina C, Duarte MCT, Antunes AEC, et al. Encapsulated thyme (Thymus vulgaris) essential oil used as a natural preservative in bakery product. Food Res Int. 2017;96:154–60.
20. Ed-Dra A, Nalbone L, Filali FR, Trabelsi N, El Majdoub YO, Bouchrif B, et al. Comprehensive evaluation on the use of Thymus vulgaris essential oil as natural additive against different serotypes of Salmonella enterica. Sustainability. 2021;13(8):4594.
21. Diniz-Silva HT, Batista de Sousa J, da Silva Guedes J, do Egypto Queiroga RdC R, Madruga MS, Tavares JF, et al. A synergistic mixture of Origanum vulgare L. and Rosmarinus officinalis L. essential oils to preserve overall quality and control Escherichia coli O157:H7 in fresh cheese during storage. Lebensm Wiss Technol. 2019;112:107781.
22. Al Zuhairi JJMJ, Jookar Kashi F, Rahimi-Moghaddam A, Yazdani M. Antioxidant, cytotoxic and antibacterial activity of Rosmarinus officinalis L. essential oil against bacteria isolated from urinary tract infection. Eur J Intern Med. 2020;38:101192.
23. Das A, Dey S, Sahoo RK, Sahoo S, Subudhi E. Antibiofilm and antibacterial activity of essential oil bearing Zingiber officinale Rosc. (ginger) rhizome against multi-drug resistant isolates. J Essent Oil Bear Plants. 2019;22(4):1163–71.
24. Risaliti L, Kehagia A, Daoultzi E, Lazari D, Bergonzi MC, Vergkizi-Nikolakaki S, et al. Liposomes loaded with Salvia triloba and Rosmarinus officinalis essential oils: in vitro assessment of antioxidant, antiinflammatory and antibacterial activities. J Drug Delivery Sci Technol. 2019;51:493–8.
25. Simirgiotis MJ, Burton D, Parra F, López J, Muñoz P, Escobar H, et al. Antioxidant and antibacterial capacities of Origanum vulgare L. essential oil from the arid andean region of Chile and its chemical characterization by GC-MS. Metabolites. 2020;10(10):414.
26. da Silva GS, de Souza ME, Quatrin PM, Klein B, Wagner R, Gündel A, et al. Nanoemulsions containing Cymbopogon flexuosus essential oil: development, characterization, stability study and evaluation of antimicrobial and antibiofilm activities. Microb Pathog. 2018;118:268–76.
27. Daglia M. Polyphenols as antimicrobial agents. Curr Opin Biotechnol. 2012;23(2):174–81.
28. Singla RK, Dubey AK, Garg A, Sharma RK, Fiorino M, Ameen SM, et al. Natural polyphenols: chemical classification, definition of classes, subcategories, and structures. J AOAC Int. 2019;102(5):1397–400.
29. Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients. 2010;2(12):1231–46.
30. Shah A, Smith DL. Flavonoids in agriculture: chemistry and roles in, biotic and abiotic stress responses, and microbial associations. Agronomy. 2020;10(8):1209.
31. Robert JK. Flavonoids. Chemistry, biochemistry and applications. Econ Bot. 2007;61(1):101.
32. Górniak I, Bartoszewski R, Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev. 2019;18(1):241–72.

33. Cosme P, Rodríguez AB, Espino J, Garrido M. Plant phenolics: bioavailability as a key determinant of their potential health-promoting applications. Antioxidants. 2020;9(12):1263.
34. Tsuchiya H. Membrane interactions of phytochemicals as their molecular mechanism applicable to the discovery of drug leads from plants. Molecules. 2015;20(10):18923–66.
35. Ma Y, Ding S, Fei Y, Liu G, Jang H, Fang J. Antimicrobial activity of anthocyanins and catechins against foodborne pathogens Escherichia coli and Salmonella. Food Control. 2019;106:106712.
36. Biharee A, Sharma A, Kumar A, Jaitak V. Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. Fitoterapia. 2020;146:104720.
37. Ozma MA, Khodadadi E, Pakdel F, Kamounah FS, Yousefi M, Yousefi B, et al. Baicalin, a natural antimicrobial and anti-biofilm agent. J Herb Med. 2021;27:100432.
38. Vandeputte OM, Kiendrebeogo M, Rasamiravaka T, Stévigny C, Duez P, Rajaonson S, et al. The flavanone naringenin reduces the production of quorum sensing-controlled virulence factors in Pseudomonas aeruginosa PAO1. Microbiology (Reading). 2011;157(7):2120–32.
39. Vikram A, Jayaprakasha GK, Jesudhasan PR, Pillai SD, Patil BS. Suppression of bacterial cell–cell signalling, biofilm formation and type III secretion system by citrus flavonoids. J Appl Microbiol. 2010;109(2):515–27.
40. Ng KR, Lyu X, Mark R, Chen WN. Antimicrobial and antioxidant activities of phenolic metabolites from flavonoid-producing yeast: potential as natural food preservatives. Food Chem. 2019;270:123–9.
41. Wang S, Yao J, Zhou B, Yang J, Chaudry MT, Wang M, et al. Bacteriostatic effect of quercetin as an antibiotic alternative in vivo and its antibacterial mechanism in vitro. J Food Prot. 2018;81(1):68–78.
42. Osonga FJ, Akgul A, Miller RM, Eshun GB, Yazgan I, Akgul A, et al. Antimicrobial activity of a new class of phosphorylated and modified flavonoids. ACS Omega. 2019;4(7):12865–71.
43. Wang G, Mohammadtursun N, Lv Y, Zhang H, Sun J, Dong J. Baicalin exerts anti-airway inflammation and anti-Remodelling effects in severe stage rat model of chronic obstructive pulmonary disease. Evid Based Complement Alternat Med. 2018;2018:7591348.
44. Li C, Huang P, Wong K, Xu Y, Tan L, Chen H, et al. Coptisine-induced inhibition of Helicobacter pylori: elucidation of specific mechanisms by probing urease active site and its maturation process. J Enzyme Inhib Med Chem. 2018;33(1):1362–75.
45. Wan B, Zhang Q, Tao J, Zhou A, Yao Y-F, Ni J. Global transcriptional regulation by H-NS and its biological influence on the virulence of Enterohemorrhagic Escherichia coli. Gene. 2016;588(2):115–23.
46. Levy J, Boyer RR, Neilson AP, O'Keefe SF, Chu HSS, Williams RC, et al. Evaluation of peanut skin and grape seed extracts to inhibit growth of foodborne pathogens. Food Sci Nutr. 2017;5(6):1130–8.
47. Calzada F, Juárez T, García-Hernández N, et al. Antiprotozoal, antibacterial and antidiarrheal properties from the flowers of Chiranthodendron pentadactylon and isolated flavonoids. Pharmacogn Mag. 2017;13(50):240–4.
48. Silva S, Costa EM, Mendes M, Morais RM, Calhau C, Pintado MM. Antimicrobial, antiadhesive and antibiofilm activity of an ethanolic, anthocyanin-rich blueberry extract purified by solid phase extraction. J Appl Microbiol. 2016;121(3):693–703.
49. Liu X, Ma B, Tan H, Jian S, Lv Z, Chen P, et al. Utilization of turmeric residue for the preparation of ceramic foam. J Clean Prod. 2021;278:123825.
50. Alagawany M, Farag MR, Abdelnour SA, Dawood MAO, Elnesr SS, Dhama K. Curcumin and its different forms: a review on fish nutrition. Aquaculture. 2021;532:736030.
51. Zorofchian Moghadamtousi S, Abdul Kadir H, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A review on antibacterial, antiviral, and antifungal activity of curcumin. Biomed Res Int. 2014;2014:186864.
52. Zheng D, Huang C, Huang H, Zhao Y, Khan MRU, Zhao H, et al. Antibacterial mechanism of curcumin: a review. Chem Biodivers. 2020;17(8):e2000171.

53. Silva AC, PDdF S, JTdP S, Leimann FV, Bracht L, Gonçalves OH. Impact of curcumin nanoformulation on its antimicrobial activity. Trends Food Sci Technol. 2018;72:74–82.
54. Adamczak A, Ożarowski M, Karpiński TM. Curcumin, a natural antimicrobial agent with strain-specific activity. Pharmaceuticals (Basel). 2020;13(7):153.
55. Teow S-Y, Liew K, Ali SA, Khoo AS-B, Peh S-C. Antibacterial action of curcumin against Staphylococcus aureus: a brief review. J Trop Med. 2016;2016:2853045.
56. Sharma G, Raturi K, Dang S, Gupta S, Gabrani R. Combinatorial antimicrobial effect of curcumin with selected phytochemicals on Staphylococcus epidermidis. J Asian Nat Prod Res. 2014;16(5):535–41.
57. Yun DG, Lee DG. Antibacterial activity of curcumin via apoptosis-like response in Escherichia coli. Appl Microbiol Biotechnol. 2016;100(12):5505–14.
58. Neyestani Z, Ebrahimi SA, Ghazaghi A, Jalili A, Sahebkar A, Rahimi HR. Review of antibacterial activities of curcumin against pseudomonas aeruginosa. Crit Rev Eukaryot Gene Expr. 2019;29(5):377–85.
59. Ahmadi Z, Mohammadinejad R, Ashrafizadeh M. Drug delivery systems for resveratrol, a non-flavonoid polyphenol: emerging evidence in last decades. J Drug Delivery Sci Technol. 2019;51:591–604.
60. Dadi PK, Ahmad M, Ahmad Z. Inhibition of ATPase activity of Escherichia coli ATP synthase by polyphenols. Int J Biol Macromol. 2009;45(1):72–9.
61. Nøhr-Meldgaard K, Ovsepian A, Ingmer H, Vestergaard M. Resveratrol enhances the efficacy of aminoglycosides against Staphylococcus aureus. Int J Antimicrob Agents. 2018;52(3):390–6.
62. Hwang D, Lim Y-H. Resveratrol antibacterial activity against Escherichia coli is mediated by Z-ring formation inhibition via suppression of FtsZ expression. Sci Rep. 2015;5:10029.
63. Paulo L, Ferreira S, Gallardo E, Queiroz JA, Domingues F. Antimicrobial activity and effects of resveratrol on human pathogenic bacteria. World J Microbiol Biotechnol. 2010;26(8):1533–8.
64. Mahady GB, Pendland SL. Resveratrol inhibits the growth of helicobacter pylori in vitro. Am J Gastroenterol. 2000;95(7):1849.
65. Martini S, Bonechi C, Rossi C, Figura N. Increased susceptibility to resveratrol of Helicobacter pylori strains isolated from patients with gastric carcinoma. J Nat Prod. 2011;74(10):2257–60.
66. Khan F, Bamunuarachchi NI, Tabassum N, Kim Y-M. Caffeic acid and its derivatives: antimicrobial drugs toward microbial pathogens. J Agric Food Chem. 2021;69(10):2979–3004.
67. Kwon YI, Apostolidis E, Labbe RG, Shetty K. Inhibition of Staphylococcus aureus by phenolic phytochemicals of selected clonal herbs species of Lamiaceae family and likely mode of action through proline oxidation. Food Biotechnol. 2007;21(1):71–89.
68. Luís Â, Silva F, Sousa S, Duarte AP, Domingues F. Antistaphylococcal and biofilm inhibitory activities of gallic, caffeic, and chlorogenic acids. Biofouling. 2014;30(1):69–79.
69. Pakravan N, Shayani-Jam H, Beiginejad H, Tavafi H, Paziresh S. A green method for the synthesis of novel spiro compounds: enhancement of antibacterial properties of caffeic acid through electrooxidation in the presence of barbituric acid derivatives. J Electroanal Chem. 2019;848:113286.
70. Alves MJ, Ferreira ICFR, Froufe HJC, Abreu RMV, Martins A, Pintado M. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. J Appl Microbiol. 2013;115(2):346–57.
71. Karpiński TM, Szkaradkiewicz AK. Bacteriocins. In: Caballero B, Finglas PM, Toldrá F, editors. Encyclopedia of food and health. Oxford: Academic; 2016. p. 312–9.
72. Benítez-Chao DF, León-Buitimea A, Lerma-Escalera JA, Morones-Ramírez JR. Bacteriocins: an overview of antimicrobial, toxicity, and biosafety assessment by in vivo models. Front Microbiol. 2021;12:630695.
73. Juturu V, Wu JC. Microbial production of bacteriocins: latest research development and applications. Biotechnol Adv. 2018;36(8):2187–200.

74. Małaczewska J, Kaczorek-Łukowska E. Nisin—a lantibiotic with immunomodulatory properties: a review. Peptides. 2021;137:170479.
75. Rivas FP, Cayré ME, Campos CA, Castro MP. Natural and artificial casings as bacteriocin carriers for the biopreservation of meats products. J Food Saf. 2018;38(1):e12419.
76. Weslie T, Felixius V, Amala Z, Shofinita D. Pediocin and grape seed extract as antimicrobial agents in nanocellulose biobased food packaging: a review. IOP Conf Ser: Mater Sci Eng. 2021;1143(1):012037.
77. Sun Z, Wang X, Zhang X, Wu H, Zou Y, Li P, et al. Class III bacteriocin Helveticin-M causes sublethal damage on target cells through impairment of cell wall and membrane. J Ind Microbiol Biotechnol. 2018;45(3):213–27.
78. Mo X, Peng X, Liang X, Fang S, Xie H, Chen J, et al. Development of antifungal gelatin-based nanocomposite films functionalized with natamycin-loaded zein/casein nanoparticles. Food Hydrocoll. 2021;113:106506.
79. Nottagh S, Hesari J, Peighambardoust SH, Rezaei-Mokarram R, Jafarizadeh-Malmiri H. Effectiveness of edible coating based on chitosan and Natamycin on biological, physico-chemical and organoleptic attributes of Iranian ultra-filtrated cheese. Biologia. 2020;75(4):605–11.
80. Delves-Broughton J. 6 – natural antimicrobials as additives and ingredients for the preservation of foods and beverages. In: Baines D, Seal R, editors. Natural food additives, Ingredients and Flavourings. Woodhead Publishing; 2012. p. 127–61.
81. Patil A, Lakhani P, Majumdar S. Current perspectives on natamycin in ocular fungal infections. J Drug Delivery Sci Technol. 2017;41:206–12.
82. Streekstra H, Verkennis AEE, Jacobs R, Dekker A, Stark J, Dijksterhuis J. Fungal strains and the development of tolerance against natamycin. Int J Food Microbiol. 2016;238:15–22.
83. Meena M, Prajapati P, Ravichandran C, Sehrawat R. Natamycin: a natural preservative for food applications—a review. Food Sci Biotechnol. 2021;30(12):1481–96.
84. Ben Said L, Gaudreau H, Dallaire L, Tessier M, Fliss I. Bioprotective culture: a new generation of food additives for the preservation of food quality and safety. Ind Biotechnol. 2019;15(3):138–47.
85. Rodrigues FJ, Cedran MF, Bicas JL, Sato HH. Reuterin-producing Limosilactobacillus reuteri: optimization of in situ reuterin production in alginate-based filmogenic solutions. Curr Res Food Sci. 2021;4:926–31.
86. Schaefer L, Auchtung TA, Hermans KE, Whitehead D, Borhan B, Britton RA. The antimicrobial compound reuterin (3-hydroxypropionaldehyde) induces oxidative stress via interaction with thiol groups. Microbiology (Reading, England). 2010;156(Pt 6):1589–99.
87. Zeng Z, Dank A, Smid EJ, Notebaart RA, Abee T. Bacterial microcompartments in food-related microbes. Curr Opin Food Sci. 2022;43:128–35.
88. Gänzle MG. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. Curr Opin Food Sci. 2015;2:106–17.
89. Khorshidian N, Khanniri E, Koushki MR, Sohrabvandi S, Yousefi M. An overview of antimicrobial activity of lysozyme and its functionality in cheese. Front Nutr. 2022;9:833618.
90. Garajová K, Balogová A, Dušeková E, Sedláková D, Sedlák E, Varhač R. Correlation of lysozyme activity and stability in the presence of Hofmeister series anions. Biochim Biophys Acta Proteins Proteom. 2017;1865(3):281–8.
91. Renzone G, Novi G, Scaloni A, Arena S. Monitoring aging of hen egg by integrated quantitative peptidomic procedures. Food Res Int. 2021;140:110010.
92. Elenska PP, Dimitrov IL. Influence of rapid cooling on crystal nucleation in lysozyme crystallization solutions of low supersaturation. Phase Transit. 2021;94(12):935–44.
93. Garcia HS, López-Hernandez A, Hill CG. 4.47 – enzyme technology – dairy industry applications. In: Moo-Young M, editor. Comprehensive biotechnology. 2nd ed. Burlington: Academic Press; 2011. p. 567–74.

94. Özer B. Natural anti-microbial systems/lactoperoxidase and lactoferrin. In: Batt CA, Tortorello ML, editors. Encyclopedia of food microbiology. 2nd ed. Oxford: Academic; 2014. p. 930–5.
95. Sharma S, Singh AK, Kaushik S, Sinha M, Singh RP, Sharma P, et al. Lactoperoxidase: structural insights into the function,ligand binding and inhibition. Int J Biochem Mol Biol. 2013;4(3):108–28.
96. Lara-Aguilar S, Alcaine SD. Lactose oxidase: a novel activator of the lactoperoxidase system in milk for improved shelf life. J Dairy Sci. 2019;102(3):1933–42.
97. Al-Baarri AN, Damayanti NT, Legowo AM, Tekiner İH, Hayakawa S. Enhanced antibacterial activity of lactoperoxidase–thiocyanate–hydrogen peroxide system in reduced-lactose milk whey. Int J Food Sci. 2019;2019:8013402.
98. Iglesias-Figueroa BF, Espinoza-Sánchez EA, Siqueiros-Cendón TS, Rascón-Cruz Q. Lactoferrin as a nutraceutical protein from milk, an overview. Int Dairy J. 2019;89:37–41.
99. Rascón-Cruz Q, Espinoza-Sánchez EA, Siqueiros-Cendón TS, Nakamura-Bencomo SI, Arévalo-Gallegos S, Iglesias-Figueroa BF. Lactoferrin: a glycoprotein involved in immunomodulation, anticancer, and antimicrobial processes. Molecules. 2021;26(1):205.
100. Sharma S, Barkauskaite S, Jaiswal AK, Jaiswal S. Essential oils as additives in active food packaging. Food Chem. 2021;343:128403.
101. Liu Y, Cai Y, Jiang X, Wu J, Le X. Molecular interactions, characterization and antimicrobial activity of curcumin–chitosan blend films. Food Hydrocoll. 2016;52:564–72.
102. Kalaycıoğlu Z, Torlak E, Akın-Evingür G, Özen İ, Erim FB. Antimicrobial and physical properties of chitosan films incorporated with turmeric extract. Int J Biol Macromol. 2017;101:882–8.
103. Wu C, Zhu Y, Wu T, Wang L, Yuan Y, Chen J, et al. Enhanced functional properties of biopolymer film incorporated with curcurmin-loaded mesoporous silica nanoparticles for food packaging. Food Chem. 2019;288:139–45.

Chapter 4
Natural Colorants

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4.1 Introduction

Natural colorants are an alternative to substitute synthetic ones in food products. The use of natural colorants in food formulations reduce the possible toxicity and allergenic characteristic associated with the use of synthetic colorants. Furthermore, the use of natural colorants can increase food acceptance, since consumers demand healthier food products with a clean labels, over the recent years [1, 2].

Several natural pigments can be used as food colorants and/or preservative, the most common natural pigments are anthocyanins, betalains, carotenoids, annatto, β-carotene, lycopene, lutein, paprika, carminic acid, chlorophylls, and curcumin [1, 2]. These pigments can be obtained from algae, fruit, vegetables, and other comestible plants, fruits, and vegetables. Furthermore, most of these natural pigments also have antioxidant, antimicrobials, anti-obesity, antidiabetic, anticarcinogenic, cardiovascular protection, and neuroprotective properties and have been used extensively in pharmacology, and recently in the food industry to produce functional foods. However, the colorimetric and functional properties of natural colorants can be modified due the oxygen presence, as well as with increase of the temperature. Other factors such as light and change of the pH also can modify the colorants properties, reducing their effectiveness and limiting their application in food industry [3–5]. This review aims compressively to analyze the state of the art related to the use of natural colorants in foods, focusing especially on their sources, properties, and potential application in the food sector. Furthermore, it was discussed their approbation by international regulatory agencies.

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4.2 Main Natural Colorants

4.2.1 Anthocyanins

Anthocyanins are one of the major studied natural pigments belonging to the flavonoid family, having more than 700 derivatives of different anthocyanins. It is soluble in water, has low or no toxicity, and has different shades of color, ranging from red to blue [1, 2]. They are found in many leaves, flowers (hibiscus), vegetables (cabbage, eggplant), tubers (purple potatoes), and fruits (grapes, raspberries, blueberries, cherries, and pomegranates) which are colored red, purple, or blue [3, 4].

Chemically, anthocyanins have a glycosidic structure (sugars and active acids) linked to an aglycone (anthocyanidin). Anthocyanidins are flavyl ion structures (2-phenylbenzopyrilium) with the keto oxygen at the C1 position and are characterized by the power to produce different types of anthocyanins through glycosylation or acylation of varying sugar and phenolic or aliphatic portions [4, 5]. The main anthocyanidins that represent about 90% of all anthocyanins identified are cyanidin, pelargonidin, petunidin, peonidin, malvidin, and malvidin delphinidin [2]. The structure of the main anthocyanins can be seen in Table 4.1.

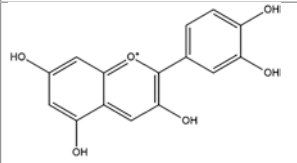
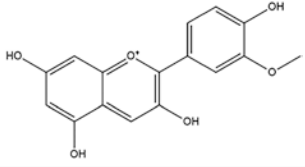
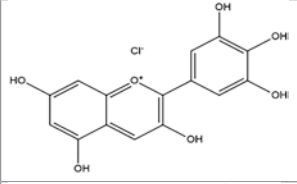
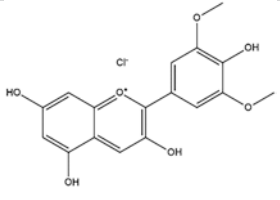
Food processing alters the accessibility and content of anthocyanins in the raw material. The bioaccessibility of anthocyanins is dependent on the food matrix and the anthocyanin structure [26]. Anthocyanins in nature are stabilized by co-pigmentation, self-association, and metal complexation [5]. As isolated anthocyanins, their stability is highly affected by temperature, oxygen, light intensity, and pH during processing, whereas they are generally stable in refrigerated storage and at acidic pH [27–29]. As it is a reactive compound, its color easily degrades because the flavyl cation reacts due to the lack of electrons. The red flavyl cation goes to a colorless carbinol alkali and eventually becomes a chalcone [30].

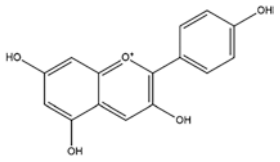
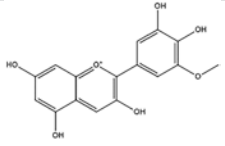
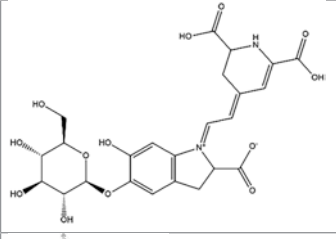
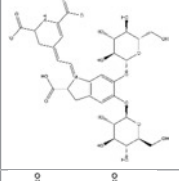
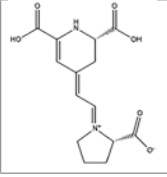
Anthocyanins are considered food additives (FAs) by the Codex Alimentarius Commission [31, 32], which has the definition of adding or restoring the color food. The standard number is 163 and varies according to the raw source, for example, grape skin (163 (ii)), extract from blackcurrant (163 (iii)), purple color from corn (163 (iv)), among others.

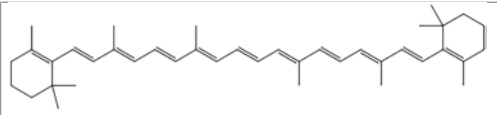
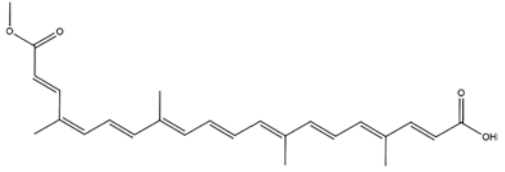
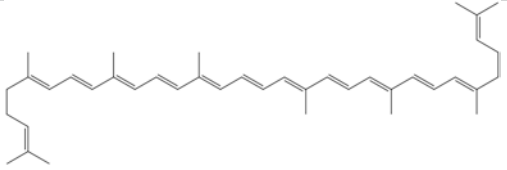
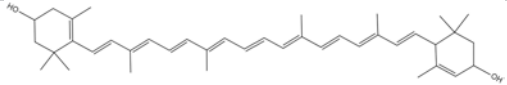
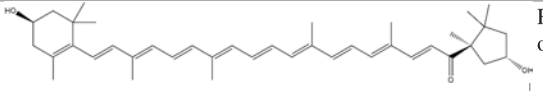
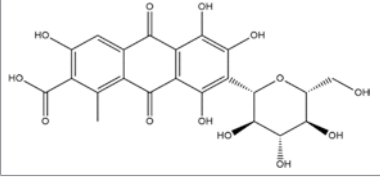
The anthocyanin color depends on several aspects such as its structure, pH, UV radiation, co-pigmentation, the concentration of the compound, presence, or absence of oxygen. Each anthocyanidin reflects a different color due to its chemical structure (Table 4.1). Cyanidin and peonidin remember reddish-purple, delphinidin, and malvidin appearing reddish-blue or purple pigment, pelargonidin, and petunidin red gives an orange hue to flowers and red to some fruits [4].

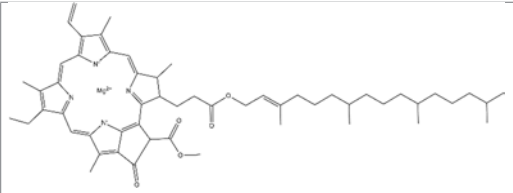
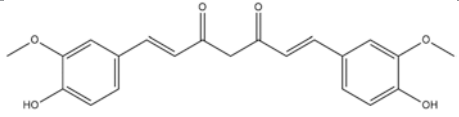
At different pH values, the ionic molecular structure of anthocyanins changes and there is a change in the tone and stability of this pigment. For example, in acidic forms, anthocyanins are found in red color. They are purple in neutral pH, and basic pH anthocyanins change to blue, green, and yellow [33–35]. Also, the anthocyanins concentration is an important factor that influence in the intensity of the extract color.

Table 4.1 Natural colorants, structure, color, primary sources, and degradation factors

Chemical classification	Pigment	Structure	Color	Primary source	Main degradation factors	Reference
Anthocyanins	Cyanidin		Red	Blackberries (*Rubus fruticosus* L.)	Heat, light, and oxygen	[6]
	Peonidin		Purple	Cranberry (*Vaccinium oxycoccus*)	Heat, light, and oxygen	[7]
	Delphinidin		Red	Uva (*Vitis vinifera*)	Heat, light, and oxygen	[8]
	Malvidin		Red-blue	Blueberry (*Vaccinium myrtillus*)	Heat, light, and oxygen	[9]

	Pelargonidin		Reddish-Orange	Strawberry (*Fragaria x ananassa*)	Heat, light, and oxygen	[10]
	Petunidin		Red	Black goji (*Lycium ruthenicum* Murr.)	Heat, light, and oxygen	[11]
Betalains	Betanin		Red-Violet	Beet (*Beta vulgaris*)	Heat, light, and oxygen	[12]
	Gomphrein		Purple	Purple Flowers (*Gomphrena globose* L.)	Heat, light, and oxygen	[13]
	Indicaxanthin		Red	Red prickly pears	Heat, light, and oxygen	[14]

Carotenoids	β-carotene		Orange-yellow	Carrot (*Daucus carota* L.)	Oxygen and light	[15–17]
	Bixin, norbixin		Red, yellow	Annatto (*Bixa orellana* L.)	Oxygen, heat, and light	[16, 18]
	Lycopene		Red	Tomato (*Solanum lypopersicum*)	Heat, oxygen, and light	[17]
	Lutein		Yellow	Marigold (*Tagetes erecta* L.)	Heat, oxygen, and light	[16, 19]
	Paprika (capsanthin)		Red-orange	Red pepper (*Capsicum annum L.*)	Heat and oxygen	[16, 20]
Carminic Acid	Carminic Acid		Red	Insects (cochonilla)	pH, light, temperature variation	[21]

Chlorophyll	Chlorophyll-a		Green	Green vegetables and microalgae	pH, heat-stable, and enzymatic reaction	[22, 23]
Curcumin	Curcumin		Yellow	*Curcuma longa* L.	Heat, light, and oxygen	[24, 25]

The color variation among anthocyanins is due to the change in hue results from a bathochromic shift, in which the light absorption band in the visible spectrum range changes from a shorter to a longer wavelength, changing the color from red to purple at acidic pH. This variation is also influenced by the presence of methoxy groups, in which the greater the presence of a methoxy group in the anthocyanin molecule, the greater the increase in blue/purple colors, such as malvidin, and the lower the concentration, the greater the increase in red color, as in pelargonidin [36].

In the literature, studies shows that anthocyanins have an high potential for application, as a natural colorant, mainly in food products and packaging. However, also have been applied in other products, such as fabrics, human hair, and sensitized solar cells [37]. In addition to dyes, the compounds may have beneficial health effects with antioxidant, anticancer, and anti-inflammatory properties [5]. As a natural food coloring and functional ingredient, the use of anthocyanins has been limited due to the low stability and interaction with other compounds in the food matrix. Therefore, alternatives such as encapsulation and adsorption have been studied to deal with these limitations [38].

4.2.2 Betalains

Betalains are nitrogenous pigments of plant origin belonging to the order Caryophyllales, formed by a central structure that is betalamic acid and substitute radicals conjugated with cyclo-Dopa (cyclo-3,4-dihydroxyphenylalanine) derived from glucosyl and amines. There are more than seventy known betalains, and they all have the same basic structure, where they are differentiated only by the modification of the radicals R1 and R2. These conjugates determine betalain classifications: red-violet betacyanins, betanidine derivatives (addition of betalamic acid and cyclo-DOPA), and yellow-orange betacyanins, resulting in the resulting from the condensation of a-amino acids or amines with betalamic acid [5, 39]. The betacyanins reported are betanin, gomphrenin, bougainvillea, and amaranthine, and betaxanthines are divided into two groups: those that are derived from amino acids and those that are derived from amines [22].

An example that for a long time was the only source of betalain, red beet (*Beta vulgaris*), but today, other sources also such as flowers, fruits (red pitaya), vegetables (chard), stems (cacti), roots, leaves, seeds, grains, and some fungi (*Amanita muscaria*, *Hygrocybe*, *and Hygrophorus*) [22–24].

Betalains are classified as FAs under code E-162 in the European Union or 73.40 by the Food and Drug Administration (FDA). The fluorescent pigmentation of betacyanins are weaker compared to betaxanthines, but their intensity is high by the carboxyl groups and low by the aromatic ring and hydroxyl groups; betaxanthines, on the other hand, have a maximum wavelength between 320 and 475 nm, which corresponds to blue light, and emission maximums between 500 and 660 nm, which corresponds to green light [25, 40].

Betalains have intrinsic and extrinsic factors that affect their stability, for both during the extraction and food processing. Its water-soluble pigments are stable from pH 3–7 and versatile for foods with low acidity and neutral content. The advantages of betalains are that colors are pH-independent and are more stable than anthocyanins in that pH range [41]. At pH values lower than 3, the anionic betalain (red color) structure is converted to cationic form (violet), showing a visible color change from red to blue violet. At pH higher 7 occurs the color change from yellow to brown due the hydrolysis of the aldimine bond and generating betalamic acid and cyclo-dopa-5-O-glycoside [42].

The bioactive compound‘s stability is affected by oxygen, light, metal ions, high water activity (aw), and temperature, limiting their use in the food industry, such as in frozen foods or in food with short shelf life. Due the biosynthesis of the compounds, betalain solutions’ must storage under low oxygen levels, low temperatures, and dark environment [40, 43, 44]. As well as others bioactive compound, several techniques to stabilize the betalain are being proposed such as encapsulation by drying methods, ionic gelling, and emulsions [45, 46], using several wall materials.

4.2.3 Carotenoids

Carotenoids are a class of lipophilic natural colorants widely distributed in nature, ranging from yellow, orange, and red. Carotenoids are tetraterpenoid pigments whose basic structure consists of 8 isoprene groups, with two C_{20} geranylgeranyl pyrophosphate molecules (composed of 4 isoprene units) linked head to head to form a C_{40} polyene chain with conjugated double bonds. The variable number of conjugated double bonds imparts carotenoids the property to absorb visible light (between 400 and 500 nm), resulting in their characteristic coloration in the yellow to red range [47, 48].

Currently, the carotenoids can be divided into two groups due to their chemical composition and structure: carotene; formed exclusively by carbons and hydrogen atoms (β-carotene, α-carotene, and lycopene); xanthophyll, in addition to carbon and hydrogen, they also contain oxygen in the structure (lutein, zeaxanthin, astaxanthin, and β-cryptoxanthin). Besides, carotenoids with a shorter carbon chain are apocarotenoids, such as bixin (annatto pigment) and crocetin (a component in saffron) [48, 49].

Carotenoids are synthesized by all photosynthetic organisms (including plants, algae, and cyanobacteria) and by some fungi, bacteria, and animals non-photosynthetic. In photosynthetic systems, carotenoids participate in light-harvesting, essential for photoprotection. In non-photosynthetic tissues and organisms, carotenoids play a role as pigments yellow, to red range. So, animals, including humans, cannot synthesize carotenoids, thus, this compound must be ingested through the diet. In foods, these compounds are present in leafy vegetables, e.g., broccoli and spinach (yellow color, unmasked when chlorophylls are degraded);

in non-leafy vegetables like carrots and pumpkin (orange color) and red color of peppers; and in fruits, e.g., watermelon and tomatoes (red colors) and orange color of mango and papaya. In addition, the red color of some fish (e.g., salmon) and crustaceans (e.g., cooked lobster, crab, and shrimp), and the yellow color of egg yolk are due to carotenoid accumulation in animal tissues. The most frequent carotenoids in food are β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene [48, 50].

Natural carotenoids from all sources food generally exist in their stable *trans* configuration, with smaller fractions in the *cis* form exhibiting less stability [48, 51]. However, the conjugated double bond system is susceptible to oxidation and isomerization during processing and storage. Oxygen, light, metal ions, and enzymes are factors that stimulate the oxidation process. The degradation product results in the formation of low molecular weight colorless compounds (volatiles) devoid of any biological activity [52].

As a natural food colorant, carotenoids can produce a range of pigments, including yellow, orange, and red, depending on the source, and are recognized as GRAS (Generally Recognized as Safe) by several regulatory agencies such as the Food and Drug Administration and the European Food Safety Authority (EFSA). Some applications of carotenoids include meat products (sausages), vegetable oils, and butter [53]. However, their use as a colorant and functional ingredient is challenging due to their water insolubility, instability, and low bioavailability. So encapsulation is a successful strategy that enhances their solubility and provides resistance against stresses during processing and digestion [47].

In addition to using carotenoids as a natural colorant, these compounds present various health benefits. The main health benefit of the carotenoids is their enzymatic conversion to retinol (Vitamin A), which is an essential micronutrient related to growth, development, immunity, epithelial barrier integrity, reproduction, and vision. Also, act as an antioxidant, anti-obesity, antidiabetic, anticarcinogenic, cardiovascular protection, and neuroprotective [47, 54].

4.2.3.1 Annatto

Annatto is the name given to the red crude extract obtained from the waxy arils that cover the seeds of the achiote tree (*Bixa Orellana L.* seeds). The achiote is a small tree or shrub native from the tropical regions including Brazil, Peru, and Mexico. However, it also grows in South and Central America [55].

Bixin and norbixin are main compounds from annatto extract, a liposoluble (bixin) and other, hydro soluble (norbixin), besides others compounds such as isobixin, beta-carotene, and lutein have been found in extract of the seeds annatto [56]. However, the bixin, is the main color compound, accounting for 80% of the total annatto pigments. Bixin ($C_{25}H_{30}O_4$) is a carotenoid of 9-cis configuration (structure present in an oxygenated carotenoid, e.g., lutein, which belongs to the xanthophyll group) and 2 carboxyl groups [55, 57].

Bixin and norbixin (E 160b) pigments exhibit high biodegradability, low toxicity, besides are stable to thermal processing, and are approved by the FDA for use in food and drinks [58]. In food industries, annatto extract is the main colorant used in the manufacture of cheese and butter, composed mainly the norbixin (a water-soluble component); this pigment confers the yellow/orange color to cheddar cheese. Also, it is applied in bakery products, snacks, and soft drinks. In general, lipid-soluble bixin is used in fatty food, whereas norbixin has been applied in food content high protein [51, 57]. However, the presence of highly conjugated π-bond structures in bixin and norbixin molecules makes them susceptible to oxidation and reduction reactions [59]. In addition, annatto being a carotenoid confers many health benefits. It acts as an antioxidant, anti-cancer, hypoglycemic, antibiotic, and anti-inflammatory [60].

4.2.3.2 β-carotene

β-carotene is the most familiar carotenoid with orange-yellow color [(E 160a (ii))] [61], obtained mainly from carrots and fungus. β-carotene has a core structure of 40 carbon atoms with 9 conjugated double bonds in the polyene chain and 2 β-ionone rings at both ends of the molecule ($C_{40}H_{56}$), providing it a lipophilic character [15, 62]. This compound can be found in various fruits and vegetables with a wide range of colors, from red to yellow, for example, in dehydrated red peppers (42.9 mg/100 g), dehydrated or raw carrot (33.95 and 8.28 mg/100 g, respectively), and raw grape leaves (16.19 mg/100 g) [63].

β-carotene naturally occurring in raw fruits and vegetables in the trans-isomers chemical form [15]. However, cis- β-carotene such as 9 cis-, 13 cis-, and 15 cis-β-carotene were found in marine microalgae species [64]. In particular, the natural 9 cis- β-carotene has been showed good results for the diseases, such as atherosclerosis, psoriasis, and inhibiting atherogenesis and retinitis pigmentosa [65].

In addition, β-carotene is the main precursor of vitamin A, resulting from the two β-ionone rings, and plays a significant role in human health [66]. It acts as an antioxidant and inhibits lipid peroxidation, shows a protective effect against cancer, cardiovascular diseases, and slows down the process of aging [51, 64].

Currently, β-carotene is one of the most exploited carotenoids used to develop functional foods, cosmetics and health-related products, and medicine [15]. However, the highly unsaturated chemical composition makes the pigment prone to oxidation in the presence of light, temperature, and metal ions, resulting in significant loss of pigment and reduction in bioactivity [51]. Encapsulation is a solution to address these limiting factors because nano or microcapsules delivery systems can improve the stability, dispersity, and bioavailability of bioactive compounds within the target food matrix [67].

4.2.3.3 Lycopene

Lycopene is a red-colored carotenoid (E 160d) with a molecular structure of $C_{40}H_{56}$, responsible for the red color of some fruits like pink grapefruit, red guava, watermelon, papaya, and is mainly present in tomatoes (*Solanum lycopersicum*) [68, 69]. Found predominantly as an all-trans isomer, but the isomers of 5-cis, 9-cis, 13-cis, and 15-cis can also was identified. The lycopene cis isomer is more absorved in human orgor body anism. This cis-trans isomerization occurs due to acidity, oxygen, heat, and light [70]. When humans ingest high lycopene content brings several health benefits due the antioxidant effect: cardioprotective, antihypercholesterolemic, antidiabetic, and anticancer. However, as nutraceutical or in a food matrix, some difficulties must be overcome, high lipophilicity and solubility in aqueous solvents, problems with stability, and thermal degradation [68].

4.2.3.4 Lutein

Lutein ($C_{40}H_{56}O_2$) is a naturally occurring fat-soluble carotenoid classified as functional xanthophyll hydroxy. This compound is abundant in vegetable and animal sources such as dark-green leafy vegetables, flowers, fruits, and egg yolks. Due to its colorant power, lutein is classified as a natural food colorant (INS 161b or E-161b) and has important biological activities such as antioxidant, anti-inflammatory, and anticancer activities. Its structure is characterized by a long carbon chain with alternating single and double carbon–carbon bonds with attached methyl side groups, according to Table 4.1 [71–75].

Its technological application has limitations, such as sensitivity to environmental factors processing and storage conditions such as heat, light, oxygen, pH, temperature, water activity, water peroxides, and lipoxygenase [76]. Different stabilization application methods improve lutein bioavailability and promote various technological applications, such as spray drying encapsulation, freeze-drying, nanoemulsions, liposomes, electrostatic complexation, and assembly, among others [38, 76–81].

Lutein esters can be applied as colorants in baked goods, dairy products, beverages, instant cereals, frozen drinks, condiments, and sweets. However, lutein esters are easily degraded due to multiple unsaturated double bonds during processing and storage [82].

4.2.3.5 Other Carotenoids

The carotenoids: capsanthin and capsorubin are presents in paprika (red pepper) of the genus *Capsicum annum L.*, Solanaceae family. Paprika, is widely used as a food ingredient, mainly as a pigment (red color) (E 160c), associated with the presence of carotenoids [83]. Paprika is native to the tropical and humid regions of Central and South America. It is widely cultivated in Brazil, Mexico, Peru, and Bolivia. South Korea and Japan have high daily food consumption of paprika [84]. The

red-orange color of paprika (*Capsicum annuum L.*) is due to the presence of the carotenoids: capsanthin and capsorubin. In this sense, capsanthin is mainly responsible for the red color, representing 40–60% of the total carotenoids in different varieties. Other carotenoids in red and orange bell peppers are β-carotene, β-cryptoxanthin, and zeaxanthin. In mature pepper fruits, the total carotenoid contents showed great variability ranging from 0.69 to 30 $mg.g^{-1}$ dry weight or 15 to 320 mg. 100 g^{-1} fresh weight, found in the pericarp and placenta [18, 84].

Paprika is traditionally used to impart pungency, color, and taste attributes in meat products, soups, sauces, and snacks. In meat products, color is improved due to the intense red color. Other characteristics are currently considered in the food industries, such as antimicrobial or antioxidant activities [18].

4.2.4 Carminic Acid

Carminic acid (7-α-$_D$-glycopyranosyl-9,10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-2 anthracenecarboxylic acid) is a water-soluble colorant extracted from insects, females of the species *Dactylopius coccus* Costa (cochonilla). These insects are found in Peru, Mexico, and the Canary Islands [85]. Carminic acid has a molecular structure composed of an anthraquinone chromophore linked to glucose, and a carboxyl group, resulting in light stability and low toxicity [86]. Its color varies according to the pH of the medium; at acidic pH, its color is orange; at slightly acidic to neutral pH, it is red, and at basic pH, the color is violet [87]. Despite having good stability in the presence of light, there is still vulnerability to photodegradation [88], thermal variations, and acidic pH [89] when used in its pure form. The extraction and purification of carminic acid are dependent on several steps, making the process complex, laborious, expensive, and dependent on several variables [90].

Carminic acid is widely used in food, medicine, and cosmetics. In Parma, Italy, the EFSA, FDA, and the USA require the presence of the information "cochineal extract" on the food label if the content is >1.8% or the carmine coloring has >50% carminic acid content. Its code as a food additive is E-120 [86, 91, 92].

4.2.5 Chlorophylls

Chlorophylls, the pigments responsible for green coloration in nature, are cyclic tetrapyrroles carrying a characteristic isocyclic five-membered ring with a function during photosynthesis [19]. Its structure is composed of a magnesium (Mg^{2+}) molecule linked to the center of a structure containing the porphyrin macrocycle that consists of four pyrrole rings. The phytol chain's side chain is strongly hydrophobic and attached to the porphyrin ring [93]. This component is fat-soluble and mainly

extracted in a non-polar or organic solvent. The various organic solvent used to extract chlorophyll-a, such as acetone, methanol, ethanol, and chloroform [94].

Nowadays, solvents and green extraction techniques have been used to extract chlorophyll. Green solvent-based extraction using 2,3-butanediol demonstrated high yield and antioxidant activity with reduced specific energy consumption. Extraction yield. 2,3- butanediol and isopropyl alcohol exhibited the highest chlorophyll extraction yields, that is, more than 70%. The extraction yields of chlorophyll-a using ethanol, ethyl lactate, and methanol were 49%, 48%, and 36%, respectively. Acetone, 1,3-butanediol and 1,3-propanediol extracted 32%, 37%, and 16% of the chlorophyll a, respectively [95]. Other techniques such as supercritical extraction, ultrasound-assisted extraction, and extraction with ionic liquids also showed extraction yields above 70%, in addition to maintaining the stability and biological activity of chlorophyll [96–98].

Chlorophyll is defined as a food additive that adds or restores color in a food [32]. Currently, this pigment is classified according to the international numbering system (INS) or the standard number in European Commission (E). In both cases, their numbering is the same, being 141 for chlorophylls and chlorophyllins, copper complexes (INS-141 or E-141), 141 (i) for chlorophylls, copper complexes; 141 (ii) chlorophyllins, copper complexes, potassium, and sodium salts [32, 99].

Copper salts are added to preserve the green color to form a chelated and more stable version of chlorophyll. Copper chlorophyllin is produced by the manufacturing process, including replacing the magnesium ligand with Cu2+, yielding a more stable product than the parent chlorophyll [100]. In addition to increasing the chemical stability of the component, this practice also improves its thermal stability, which helps in its application as a food colorant [19]. Among the green food colorants, E-141ii (also known as copper complexes of chlorophyllins) is the most used in food technologies due to its hydrophilic character and high green color stability [101]. Table 4.1 shows the general structure of the chlorophyll, chlorophyllins, and copper complexes.

4.2.6 Curcumin

Curcumin is a water-insoluble polyphenol with antioxidant, anti-inflammatory, antimutagenic, anti-Alzheimer, anticancer, antimicrobial, neuroprotective, cardioprotective activities. In addition to its nutraceutical benefits, curcumin (E100 – INS 100) is a natural yellow colorant that can replace artificial colorants such as tartrazine (E102) [16, 20, 102, 103].

Curcumin shows a low molecular weight (368.38 g/mol), a melting point of approximately 183 °C, and low water solubility (0.6 μg/mL). Its chemical structure comprises two methoxyphenyl rings, which are symmetrically linked in conjugation through the β-diketone portion, which confers exciting properties. The β-diketone structure is responsible for the intramolecular transfer of the hydrogen atom that leads this molecule to keto-enol tautomerism (Table 4.1). At pH 3–7, the

Table 4.2 Natural colorants added to food matrix

Source	Natural pigment	Food product	Main results	Reference
Figs, blackthorns	Anthocyanin: Cyanidin	Donuts, Dairy pastry	Anthocyanin extracts from fig and blackthorn were incorporated into donut icing and dairy pastry as colorants. Antioxidant and antimicrobial activities significantly increased in both extracts. The donuts topping presented less firmness and consistency and the "beijinho" presented greater softness, in both. Nutritionally there were no significant differences, but there were in the rheological properties. In 24 h, the blackthorn extract topping donuts lost color considerably, while the fig extract was stable	[113]
Blue-corn	Anthocyanin: Cyanidin	Polvorones	Anthocyanins were used to enrich commercial wheat flour with polvorones. The addition of blue corn flour did not change bromatological aspects but increased the content of phenolic compounds (6.1 times) and antioxidants (27.9 times) compared to control samples. The flour enriched with anthocyanins showed greater softness and general acceptability and color and flavor	[114]
Jabuticaba, cochineal	Anthocyanins, Carminic acid	Fresh sausage	Microencapsulated jabuticaba and cochineal carmine were added to fresh sausage as natural dyes. Jabuticaba reduced the lipid oxidation of sausages during 15 days of storage at 1 °C compared to the control sample and to carmine. The color intensity of carminic acid was higher than that of sausage with jabuticaba extract	[115]

(continued)

Table 4.2 (continued)

Source	Natural pigment	Food product	Main results	Reference
Red pitaya	Betalains: Betacyanins	Intelligent packaging	Betacyanins were added to films with different polysaccharides (chitosan, k-carrageenan and locust bean gum) and polyvinyl alcohol (PVA). Betacyanins increased the antioxidant capacity and pH/ammonia sensitivity of the films. The type of polysaccharide influenced the intensity of color, intermolecular interactions, antioxidant capacity and sensitivity to ammonia and pH of betacyanins. The film with locust bean gum/PVA/red pitaya pulp extract showed the highest color and antioxidant stability, being the most suitable for monitoring shrimp freshness	[116]
Red beet	Betalains: Betanin	Intelligent packaging	The addition of betanin dye considerably reduced the transparency and hydrophilicity of the surface of the gelatin films. The higher the dye concentration, the greater the solubility of the films. Films containing betalains showed better color retention capacity, regardless of concentration, compared to films containing curcumin and anthocyanins. The color of the films varied from reddish-purple to violet. Film with promising capabilities for food freshness monitoring application	[117]

(continued)

Table 4.2 (continued)

Source	Natural pigment	Food product	Main results	Reference
β-carotene commercial	Carotenoids: β-carotene	Mayonnaise	β-carotene loaded lipid particles were used as a colorant in mayonnaise. Sample with 5 mg of β-carotene/25 g of commercial mayonnaise shows the best color, resembling homemade mayonnaise. A significant difference was obtained between the control and the colored sample. However, during 15 days of storage, the parameters L* and b* did not show statistically significant differences with time. In contrast, parameter a* showed an increase of the red color intensity	[118]
Carrot	Carotenoid	Tapioca pancakes	Cassava gum fortified with carrot carotenoid microparticles was used to prepare tapioca pancakes. Due to the heat process, low levels of carotenoid loss (22%) were observed upon preparing tapioca "pancakes" using the fortified cassava gum. ΔE value of 8.79 for the cassava gum fortified by microparticles suggests that the color change was classified as very distinct after storage 30 days at 10 °C in dark conditions	[119]
Mandarin epicarp (*Citrus reticulata*)	Carotenoids	Cakes and bread	Carotenoids extract from mandarin epicarp were used as a natural coloring additive with the potential to reduce the use of tartrazine in bakery products such as cakes and bread. The cake had a lower concentration of carotenoids regarding the bread, possibly because of the temperatures used. The overall color change (ΔE) was greater in the crust than in the crumb in the two products. For the ΔE in the crumb, it was observed that the values were >2	[120]

(continued)

Table 4.2 (continued)

Source	Natural pigment	Food product	Main results	Reference
Annatto seeds (*Bixa orellana L.*)	Carotenoids: norbixin	Isotonic drinks	Norbixin microcapsules were added to isotonic tangerine soft drinks. Isotonic drinks with microcapsules presented a more intense orange color and lower color loss during storage under accelerated conditions (heat and light) than the control sample (added with non-encapsulated norbixin)	[121]
Marigold (*Tagetes erecta* L.)	Lutein	Sheep milk yogurt	Lutein was added to yogurt manufactured from sheep milk. Lutein did not influence fermentation patterns, but post acidification was observed, mainly in groups with the highest lutein concentrations. The yogurt color obtained a yellow color due to the addition of the natural colorant lutein, showing Hue Angle values around 90.51° ± 0.42. At 7.8 mg of LT per portion (200 mL), which has reached the minimum daily intake recommended by many researchers for health benefits	[122]
Lyophilized biomass of *Muriellopsis sp*	Lutein	Mayonnaise	Mayonnaise has been enriched with lutein. From the addition of the natural colorant, it was possible to obtain the color of traditional commercial mayonnaise. In 632 grams of the lutein-enriched mayonnaise, a polyphenol content of 3.63 mg GAE and an ORAC of 33.40 μmol/TE allowed for a polyphenol intake of 0.09 mg EAG/day and an antioxidant capacity of 0.79 μmol ET/day	[123]

(continued)

Table 4.2 (continued)

Source	Natural pigment	Food product	Main results	Reference
Tomato	Carotenoids: lycopene	Bread and muffin	Bread and muffins supplemented with tomato pomace (35% and 40%) have enhanced nutritional properties such as dietary fiber, vitamin C, antioxidant activity, and minerals. In addition, they presented acceptable color and sensory properties and a softer crumb texture than the control bakery products. There was an increase in a* and b* for the color parameters, while the L* values decreased	[124]
Stinging nettle (*Urtica dioica L.*)	Carotenoids: lutein and β-carotene	Egg pasta	The use of stinging nettle as a functional ingredient for enriching egg pasta provides for 11% more lutein and 55% more β-carotene than non-enriched pasta even though the cooking process produces loss phytocompounds due to temperature degradation and water boiling	[125]
Tumeric rhizomes	Curcumin	Buffer solution as simulating food and yogurt	Nano encapsulated curcumin presented the highest antioxidant potential by OxHLIA and TBARS. At pH 3.0, curcumin showed a yellow color favoring the keto-form formation. This approach encourages their application as health-promoting compounds to substitute artificial food coloring additives	[126]

(continued)

Table 4.2 (continued)

Source	Natural pigment	Food product	Main results	Reference
Tumeric rhizomes	Curcumin	Gelatin films	Curcumin has been applied as a natural colorant on fish gelatin-based films. The addition of colorants altered the color, light barrier, and wettability of the films. The addition of colorants provided films with the capacity to sense pH changes before and after immersion in a fatty food simulant. These properties establish the suitability of the films for intelligent fatty food packaging applications	[117]
Tumeric residue	Curcumin	Hydrogel coating for sausages	Turmeric residue was used to prepare light-activated antimicrobial hydrogel coatings. The coatings were applied to the surface of cooked sausages and evaluated for their ability to prevent bacterial cross-contamination. It was observed that UV-A light-exposed hydrogels coatings could inactivate more than 5 log CFU/mL of *L. innocua* after light treatments as short as 5 min. In addition, the light-activated antimicrobial activity of the hydrogel coatings was not affected by the incubation temperature	[127]
Microalgae (*Arthrospira platensis* F&M-C256, *Chlorella vulgaris* Allma, *Tetraselmis suecica* F&M-M33 and *Phaeodactylum tricornutum* F&M-M40)	Chlorophyll	Cookies	The addition of chlorophyll-rich microalgae provided innovative and stable green shades that varied, depending on the microalgae used, from a bluish-green (*A. platensis*) to a brownish-green (*P. tricornutum*). Increasing the microalgae content from 2% to 6% resulted in a significant increase ($p < 0.05$) in the total phenolic content and the cookies' antioxidant capacity	[128]

ketone form predominates, while at pH above 8, the enol form is a majority. Its industrial use is difficult to achieve due to the low water affinity, pH, and thermal instability [20, 38]. These limitations can be overcome by applying stabilization methods such as spray drying, ionic gelation, liposomes, among others [38, 104–106].

4.2.7 Other Natural Colorants

The main natural colorants already used for the food industry are anthocyanins, betalains, carotenoids, carminic acid, chlorophylls, and curcumin. Despite the noticeable advances regarding the replacement of artificial colors by natural colors, the natural blue color is still an industry challenge. The blue natural sources are limited, and this pigment is also further limited by its poor light stability and high sensitivity to heat, losing color at temperatures ≥45 °C. In addition, none of the existing natural blue colorants reach the versatility, low cost, and intensity of synthetic blues. Phycocyanin is a precursor of the blue colorant, and recently, FDA approved phycocyanin as a spirulina extract [16, 107]. *Spirulina* sp. synthesizes C-phycocyanin (phycocyanin from cyanobacteria), a blue and water-soluble pigment [108]. Examples of natural blue dye applications are blue cheese, ice cream, and dairy beverages [16, 109, 110].

4.3 Applications of Natural Food Colorants

FAs are molecules introduced in foodstuffs to carry out specific technological functions such as preservative food, improving color, taste, sweetening, and texturizing; they are added during food manufacture, complying with the regulatory criteria of each country [111].

The colorants are widely employed among the FAs because color is one of the most important sensory aspects and an essential criterion for consumer choice. During the processing and storage of food, losses of the natural color of the product are observed such as juice, cream, feed solutions, thus colorants additives are added to give it a more attractive appearance. Synthetic food additives (SFAs) are the principal colorants used by food industries. However, they are progressively substituted by a natural source, due mainly to the consumer preferences and the numerous health effects such as allergic reactions and the toxicity of the SFAs [112].

Natural colorants, derivatives from anthocyanin, betalains, carotenoids, carminic acid, chlorophylls, and curcumin, provide a wide variety of colors to use in food products. Also, play a health beneficial (antioxidant, anticancer, and anti-inflammatory). So, this topic presents the primary studies that used natural pigments to apply in food products, according to described in Table 4.2.

Anthocyanins show promising results in replacing synthetic dyes (red, pink, and orange) in dairy products. Tereucan et al. [129] added an extract from purple potato to milk and yogurt and showed high dye stability in cold storage, resembling commercial storage time using synthetic dyes. Swer et al. [130] applied anthocyanins extracted from Sohiong (*Prunus nepalensis* L.) in the processing of yogurt, syrup, and candies and observed that higher concentrations of the pigment were more acceptable in the sensory evaluation when compared to the control sample, being at the same level organoleptically. In addition, higher pigment concentrations also favored color stability in all products. Other examples of the application of anthocyanins as dyes in yogurts are Byamukama et al. [131], using anthocyanins from blackberry (*Morus rubra*) and Pires et al. [132] who incorporated natural dyes obtained from edible flowers such as *Centaurea cyanus* L. (cornflower) and *Dahlia mignon* (dahlia).

Anthocyanins also were applied in other products; for example, Sampaio et al. [133] developed a soft drink formulation with anthocyanin extract from purple (cv. *Purple*, *Violetta,* and *Kefermarkter Blaue*) and red (cv. *Rosemary*, *Red Emmalie,* and *Red Cardinal*) potatoes. Soft drinks with extracts presented good sensory and shelf life profiles than the commercial control dye E-163. Montibeller et al. [134] evaluated the stability of grape skin anthocyanins in kefir and carbonated water as a dye. They observed that the stability of the compound followed the first-order reaction kinetics and was different in the different matrices, indicating the use of dark packaging to avoid the degradation of anthocyanin. Albuquerque et al. [135] applied anthocyanin extract from jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg.) in macarons and obtained a more stable color than the commercial dye E-163 within 6-day shelf life.

In food packaging, anthocyanins are applied to active and intelligent packaging to monitor the freshness of various products due to their ability to change color at different pHs. Several works have different matrices, carbohydrates (starch, pectin), proteins (chitosan, gelatin), and other sources, with different raw materials containing the compound. Zheng et al. [136] produced two colorimetric films based on chitin and sodium alginate/gelatin containing anthocyanins from goji berries to monitor pork freshness and obtained good accuracy in colorimetric response to amine gases and good durability. Sani et al. [137] developed methylcellulose and chitin nanofiber films with anthocyanins from barberry (*Berberis vulgaris* L.) to monitor freshness in fish. The indicator changed color from red to pink and then yellow with increasing pH and consequent ammonia vapor concentration. In addition to its properties as a dye, it also acted significantly as an antioxidant and antimicrobial. An example of the combination of active/intelligent packaging is the study carried out by Wu et al. [138], who developed a film based on gellan gum and *Clitoria ternatea* extract to monitor shrimp freshness. The application had a satisfactory result in releasing the compound and acting as an antimicrobial, as in the colorimetric alteration, suggesting that this package can be used in foods. Other examples of anthocyanin sources used in intelligent packaging are butterfly pea [139], purple potato [140, 141], red cabbage [37], turmeric (*Crocus sativus* L.), and red barberry [142].

Betalains are also used as a coloring agent in food products or active and intelligent packaging; however, they are less studied than anthocyanins. Otálora et al. [143] developed gummy bears containing encapsulated betalains from (*Opuntia ficus-indica*). Betalain stability was investigated, and there was no significant loss at 4 °C for 30 days, indicating good stability under these conditions. The gummy color was bright red-purple. Moghaddas Kia et al. [144] developed gelatin/gellan-based gummy bears with red beetroot extract as a dye and observed an increase in gum surface gloss with the combined use of gellan gum and red beetroot extract. The color of the gummy was satisfactory, with low concentrations of the extract (0.3% and 0.1%) demonstrating the potential of betalain as a food coloring. Kharrat et al. [145] studied the stability of betalains from prickly pear (*Opuntia stricta*) extract in salami. The extract showed positive results in the sensory analysis of salami, a promising substitute for carminic acid or other synthetic dyes, also obtained good results as an antimicrobial and antioxidant agent. Roriz et al. [13] incorporated beetroot extract (*Gomphrena globosa* L.) into ice cream. The dye remained stable during storage (−22 °C, 60 days) and obtained similar results to commercial betalain, indicating its use as a substitute. Yang et al. [146] compared anthocyanin from grape (*Vitis vinifera*) and betalains from red beet (*Beta vulgaris*) as colorants in white currant juice with regard to storage time, color, and sensory stability. They observed that anthocyanins are more stable than betalains during storage at room temperature and 4 °C. The color of the juice became more yellow and clear, which indicates the degradation of the compound. The mixture of the two compounds did not result in greater stability, and this option is not favorable.

In packaging, we have the study carried out by Yao et al. [147] on developing antioxidant, antimicrobial, and ammonia-sensitive films using betalain-rich forage palm extract (*Opuntia ficus-indica*). They obtained good responses to the ammonia concentration (color ranging from purple to orange) containing only 2% and 3% by weight of extract and improving the functional properties. Qin et al. [148] developed an active/smart packaging incorporating betalains from red pitaya (*Hylocereus polyrhizus*) bark in starch/polyvinyl alcohol films, which 1% by weight of extract was efficient to monitoring the freshness of shrimp. The film was very sensitive to ammonia, changing from pink to yellow during 48 h in contact with the shrimp. In addition to being an indicator, betalain extract resulted in a greater light barrier antioxidant and antimicrobial properties.

Another natural colorant used in food are the carotenoids that confer colors from yellow to red. These pigments are unstable due to environmental factors such as pH, temperature, oxygen, and light. Thus, the studies used encapsulation to protect these compounds. Carotenoids from yellow bell pepper pigments were encapsulated with β-cyclodextrin, and their stability in isotonic beverages (pH: 2.9; 0.02, 0.05, and 0.06% of pigment addition) were evaluated. Lutein, zeaxanthin, α-cryptoxanthin, α-carotene, and β-carotene were the main carotenoids found. Extract added in beverages exhibited dose-dependent luminosity and redness increase but decrease in yellowness. Good results for the color stability indices were demonstrated for isotonic drinks stained with complex obtained by inclusion method compared to those stained with crude yellow pepper extract (storage 21 days) [149].

β-carotene encapsulated in a beeswax-based solid lipid particle was tested concerning their colorant power by selecting a food matrix widely appreciated and consumed (mayonnaise). The best formulation presented 5 mg of β-carotene per 25 g of mayonnaise, resembling as much as possible the appearance of homemade mayonnaise, as it might be more attractive for consumers. The color parameters were evaluated for 15 days at 6 °C, showing color stability and nutritional value maintenance after 15 days under storage [118].

Lutein was applied as a colorant to replace urucum, in the Prato cheese formulation, at 16 and 32 mg L^{-1} concentrations and did not show differences in the quality attributes such as color, pH, texture, as well as did not affect the maturation profile and sensory acceptance. Also, lutein kept stable in cheese for 60 days, thus maintaining its antioxidant capacity [150]. Lutein was also applied in feed supplementation, raw milk, and mozzarella cheese. The lutein content in raw milk increased approximately three-fold after 2 months of dietary supplementation with lutein. Most of the lutein remained in the mozzarella cheese during the cheese-making process, but part was lost to the whey, hot water, and brine. Approximately 20% of lutein was lost during the 8 weeks storage period of the mozzarella cheese [151].

The addition of lutein colorant was evaluated on the oxidative stability in yogurt for 35 days stored at 5 °C under presence and absence of light. Yogurts (120 g) with the addition of 0.5, 1.5, and 2.5 mg of lutein colorant were evaluated for the sensory acceptance, showing no differences between aroma and flavor attributes among samples. The addition of lutein also conferred oxidative stability to the yogurts. The lutein content remained stable during exposure to light, meaning that the lutein added in the yogurt was present in the product during the storage [152]. The stability of the natural colorant lutein, obtained from the biomass of the microalgae *Muriellopsis sp*, and its antioxidant activity were evaluated during the storage of mayonnaise at 5 °C for 3 months. It was observed that the addition of natural colorant maintained the commercial color of mayonnaise for 3 months and the storage period [123].

4.3.1 Colorants from Agroindustry Waste

Adding value to fruit and vegetable by-products would satisfy global demand for NFAs and reduce environmental impacts. In this context, carotenoid obtained from waste was employed in bakery products. Mehta et al. [124] investigated the effect of the carotenoids from tomato pomace, in physicochemical characteristics, and shelf-life stability of the bread and muffin products. Bread and muffin supplemented with tomato pomace showed enhance nutritional properties like dietary fiber, vitamin C, antioxidant activity, minerals, and acceptable color and sensory properties. Furthermore, increase in the shelf-life compared to control bakery products with or without preservatives. Another study evaluated ultrasound-assisted carotenoid extraction from mandarin epicarp for use as a natural coloring in two baked goods: cakes and breads to reduce tartrazine preservative used in these product [120].

Carminic acid is mainly applied to meat products during their curing with salts to give the meat color. It is already allowed in some countries and international legislation, but few studies involve the compound [153]. Recently Ongaratto et al. [154] studied the incorporation of carminic acid adsorbed into zinc hydroxide salt in mortadella to improve stability and color, resulting in the pinkish, slightly reddish color characteristic of mortadella, indicating a promising potential in the substitution of curing salts.

Chlorophyll is used to add green color to food. The natural colorant was incorporated into fresh gluten-free pasta. The addition of a platensis biomass as an ingredient resulted in doughs with an attractive appearance. The gluten-free supplemented pasta showed higher antioxidant activity than the control, good mechanical properties, and high in vitro digestibility without affecting the cooking properties of the pasta [155]. Freeze-dried *Chlorella sorokiniana* biomass rich in chlorophyll dye was incorporated into pasta. Replacing 5% flour increased protein and lipid content to 15.7 ± 0.50% and 4.1 ± 0.06%, respectively. Meanwhile, adding the microalgae Chlorella to the pasta helped increase the polyunsaturated fatty acids, chlorophyll, and carotenoids necessary for preventing foodborne diseases [156]. Emulsifiers Sucrose fatty acid ester and quillaja saponin were applied to protect the chlorophyll of green tea and vegetable juices from the bleaching process. The formation of chlorophyll nanoparticles (100 nm) caused a self-stacking to form many aggregates soluble in the aqueous emulsifier solution, suppressing the discoloration of Chl. In this way, applying nano encapsulated chlorophyll by emulsions in green drinks becomes possible [157].

Chlorophyll natural colorant can be applied as a pH-sensitive natural indicator in intelligent films to monitor the quality of foods such as fish and minimally processed green peppers [136, 158]. Despite being a colorant legally used to add green tones to foods, chlorophyll derivatives are prohibited in Europe and America for fats and oils. One of the main frauds is the application of the green dye derived from chlorophyll in olives and olive oil to return or intensify the green color of these products that are degraded due to processing.

Curcumin is a hydrophobic colorant, so it needs to undergo a modification/compatibilization process with the aqueous medium to improve stability and enable its application in hydrophilic food matrices. Curcumin nano encapsulated by solid-phase dispersion was incorporated as a coloring in yogurt. Curcumin maintained the color of the yogurt during the entire storage period (7 days at 4 °C) without causing relevant changes in the nutritional composition and fatty acid profiles. In addition, it was also possible to observe the antioxidant, anti-inflammatory, cytotoxic, and antibacterial activity [103]. An essential application of curcumin can be observed in yellowish foods such as cheese. Curcumin was used in Minas Frescal cheese as a photosensitizing agent in antimicrobial photodynamic therapy for the inactivation of pseudomonas. Inactivation of 7 log CFU/mL of *P. fluorescens* was observed at a 62.50 μg/mL concentration of curcumin solubilized in ethanol [159]. Curcumin solutions were infused into cooked oysters by vacuum to inhibit growth of total mesophilic and total psychrophilic bacteria in oysters during storage. A positive antimicrobial effect was observed through increased shelf life of cooked oysters [160].

Curcumin-loaded nanoemulsions were incorporated into a commercial salad dressing. To stabilize the emulsions, whey protein and quillaja saponin were used. The salad dressings produced were light or yellow, characteristic of classic production ingredients such as mustard, egg yolk, and riboflavin. The viscosity of the salad dressing mixtures decreased as the nanoemulsion concentration in the mixtures was increased due to the fitting into the spaces between the larger particles in the salad dressings. It was also observed greater ease of mixing the nano drops with the salad dressing, indicating that this technology can be used as a vehicle for curcumin dye and other hydrophobic bioactive compounds in commercial products [161].

Curcumin can be used with natural coloring in active packaging and intelligent packaging. Curcumin was incorporated into chitosan and polyethylene oxide films by electrospinning to monitor chicken breast freshness. The packages were stored at 4 °C for 8 days, showing the color change of the nanofiber film from bright yellow to reddish, which allowed the detection of color changes even with the naked eye of the untrained consumer [162]. Tosati et al. [163] incorporated curcumin in an edible coating based on starch and bovine gelatin, replacing the synthetic casing used in the coating of frankfurter sausage. It was possible to observe the antimicrobial effect of the natural colorant applied as active packaging in sausages stored at 5 °C for 20 days, while the uncoated sausages had a shelf life of 10 days.

Natural pigments were applied in several types of food, beverages, cereals, milk, meats, and bakery and were used in intelligent packaging. Most studies evaluate the changes in physical and chemical characteristics with the addition of these ingredients. However, few studies focus on sensory evaluation, which is essential for the consumer acceptance and purchase of food. In addition, it is essential to highlight that natural colorants are unstable due to environmental factors such as pH, temperature, oxygen, and light [38]. Thus, many studies used encapsulation to protect these compounds and used them as food ingredients.

4.4 Conclusions

This chapter summarized the importance of using natural additives in foods, their main sources, and their properties. Based on the literature, pigments such as anthocyanins, betalains, carotenoids, annatto, β-carotene, lycopene, lutein, carminic acid, chlorophylls, and curcumin can be isolated from natural sources, being used as food colorants.

Based on the reviewed literature, anthocyanins have been the most used colorants, followed by lutein, carotenoids, betalains, chlorophyll, and carminic acid. Several food products such as milk, yogurt, cheese, candies, soft drink, kefir, macarons, ice cream, mayonnaise, salami, beverages, and pasta can be produced by incorporating natural colorants. Furthermore, the use of these natural pigments can reduce the oxidation of foods during storage, increasing their shelf life. In addition, anthocyanins, betalains, and chlorophyll have been the main natural colorants used

to develop active and intelligent food packaging. The stabilization of natural colorants must be investigated aiming to increase their stability when applied in foods.

Conflicts of Interest The author declares no conflict of interest.

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References

1. Mazza G, Miniati E. Anthocyanins in fruits, vegetables, and grains. CRC Press; 2018. 327 p.
2. Wallace TC, Giusti MM. Anthocyanins—nature's bold, beautiful, and health-promoting colors. Foods. Multidisciplinary Digital Publishing Institute. 2019;8:550.
3. Li H, Deng Z, Zhu H, Hu C, Liu R, Young JC, et al. Highly pigmented vegetables: anthocyanin compositions and their role in antioxidant activities. Food Res Int. 2012;46(1):250–9.
4. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res [Internet]. 2017;61(1):1361779. Available from: https://doi.org/10.1080/16546628.2017.1361779.
5. Rodriguez-Amaya DB. Natural food pigments and colorants. Curr Opin Food Sci. 2016;7:20–6.
6. Cruz L, Basílio N, de Freitas V. Color stabilization of cyanidin-3-glucoside-based dyes by encapsulation with biocompatible PEGylated phospholipid micelles. Dyes Pigments. 2020;181(April):108592.
7. Rajan VK, Hasna CK, Muraleedharan K. The natural food colorant Peonidin from cranberries as a potential radical scavenger – a DFT based mechanistic analysis. Food Chem. 2018;262(April):184–90.
8. Gordillo B, Sigurdson GT, Lao F, González-Miret ML, Heredia FJ, Giusti MM. Assessment of the color modulation and stability of naturally copigmented anthocyanin-grape colorants with different levels of purification. Food Res Int. 2018;106(February):791–9.
9. Herrera-Balandrano DD, Chai Z, Beta T, Feng J, Huang W. Blueberry anthocyanins: an updated review on approaches to enhancing their bioavailability. Trends Food Sci Technol. 2021;118(PB):808–21.
10. Liu H, Shi C, Sun X, Zhang J, Ji Z. Intelligent colorimetric indicator film based on bacterial cellulose and pelargonidin dye to indicate the freshness of tilapia fillets. Food Packag Shelf Life. 2021;29(January):100712.
11. Tang P, Giusti MM. Black goji as a potential source of natural color in a wide pH range. Food Chem. 2018;269(February):419–26.
12. Grewal PS, Modavi C, Russ ZN, Harris NC, Dueber JE. Bioproduction of a betalain color palette in Saccharomyces cerevisiae. Metab Eng. 2018;45(December 2017):180–8.
13. Roriz CL, Barreira JCM, Morales P, Barros L, Ferreira ICFR. Gomphrena globosa L. as a novel source of food-grade betacyanins: incorporation in ice-cream and comparison with beet-root extracts and commercial betalains. Lwt. 2018;92(October 2017):101–7.

14. Ortega-Hernández E, Nair V, Serrano-Sandoval SN, Welti-Chanes J, Cisneros-Zevallos L, Jacobo-Velázquez DA. Wounding and UVB light synergistically induce the postharvest biosynthesis of indicaxanthin and betanin in red prickly pears. Postharvest Biol Technol. 2020;167(May):111247.
15. Cassani L, Marcovich NE, Gomez-Zavaglia A. Valorization of fruit and vegetables agro-wastes for the sustainable production of carotenoid-based colorants with enhanced bioavailability. Food Res Int [Internet]. 2021;152(August 2021):110924. Available from: https://doi.org/10.1016/j.foodres.2021.110924.
16. Landim Neves MI, Silva EK, Meireles MAA. Natural blue food colorants: consumer acceptance, current alternatives, trends, challenges, and future strategies. Trends Food Sci Technol [Internet]. 2021;112(September 2020):163–73. Available from: https://doi.org/10.1016/j.tifs.2021.03.023.
17. Carvalho GC, Sábio RM, Chorilli M. An overview of properties and analytical methods for lycopene in organic nanocarriers. Crit Rev Anal Chem [Internet]. 2021;51(7):674–86. Available from: https://doi.org/10.1080/10408347.2020.1763774.
18. Baenas N, Belović M, Ilic N, Moreno DA, García-Viguera C. Industrial use of pepper (Capsicum annum L.) derived products: technological benefits and biological advantages. Food Chem. 2019;274(April 2018):872–85.
19. Pérez-Gálvez A, Viera I, Roca M. Development of an accurate and direct method for the green food colorants detection. Food Res Int [Internet]. 2020;136(June):109484. Available from: https://doi.org/10.1016/j.foodres.2020.109484.
20. Leimann VF, Gonçalves OH, Sorita GD, Rezende S, Bona E, Fernandes IPM, et al. Heat and pH stable curcumin-based hydrophylic colorants obtained by the solid dispersion technology assisted by spray-drying. Chem Eng Sci [Internet]. 2019;205:248–58. Available from: https://doi.org/10.1016/j.ces.2019.04.044.
21. Guillermin D, Debroise T, Trigueiro P, de Viguerie L, Rigaud B, Morlet-Savary F, et al. New pigments based on carminic acid and smectites: a molecular investigation. Dyes Pigments. 2019;160(July 2018):971–82.
22. Slimen IB, Najar T, Abderrabba M. Correction to chemical and antioxidant properties of betalains. J Agric Food Chem. 2017;65(7):1466.
23. Moßhammer MR, Stintzing FC, Carle R. Evaluation of different methods for the production of juice concentrates and fruit powders from cactus pear. Innov Food Sci Emerg Technol. 2006;7(4):275–87.
24. Herbach KM, Maier C, Stintzing FC, Carle R. Effects of processing and storage on juice colour and betacyanin stability of purple pitaya (Hylocereus polyrhizus) juice. Eur Food Res Technol. 2007;224(5):649–58.
25. Gandía-Herrero F, Escribano J, García-Carmona F. Structural implications on color, fluorescence, and antiradical activity in betalains. Planta. 2010;232(2):449–60.
26. Cavalcanti RN, Santos DT, Meireles MAA. Non-thermal stabilization mechanisms of anthocyanins in model and food systems—an overview. Food Res Int. 2011;44(2):499–509.
27. Wrolstad RE, Culver CA. Alternatives to those artificial FD&C food colorants. Annu Rev Food Sci Technol. 2012;3:59–77.
28. Iacobucci GA, Sweeny JG. The chemistry of anthocyanins, anthocyanidins and related flavylium salts. Tetrahedron. 1983;39(19):3005–38.
29. Sui X, Sun H, Qi B, Zhang M, Li Y, Jiang L. Functional and conformational changes to soy proteins accompanying anthocyanins: focus on covalent and non-covalent interactions. Food Chem. 2018;245:871–8.
30. Markakis P. Stability of anthocyanins in foods. In: Markakis P, editor. Anthocyanins as food color. Elsevier; 1982. p. 163–80.
31. Codex Alimentarius Commission. Class names and the international numbering system for food additives. CXG 36-1989. 2008
32. Codex Alimentarius Commission. Class names and the international numbering system for food additives. CXG 36-1989. April 2008. p. 33–5. Available from: https://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/.

33. Roy S, Rhim J-W. Anthocyanin food colorant and its application in pH-responsive color change indicator films. Crit Rev Food Sci Nutr. 2021;61(14):2297–325.
34. B1kowska-Barczak A. Acylated anthocyanins as stable, natural food colorants—A review. Polish J Food Nutr Sci. 2005;14:55.
35. Castañeda-Ovando A, Pacheco-Hernández M d L, Páez-Hernández ME, Rodríguez JA, Galán-Vidal CA. Chemical studies of anthocyanins: a review. Food Chem [Internet]. 2009;113(4):859–71. Available from: https://doi.org/10.1016/j.foodchem.2008.09.001.
36. Fennema OR. Food chemistry. M. Dekker; 1961.
37. Weston M, Phan MAT, Arcot J, Chandrawati R. Anthocyanin-based sensors derived from food waste as an active use-by date indicator for milk. Food Chem. 2020;326(November 2019):127017.
38. Luiza Koop B, Nascimento da Silva M, Diniz da Silva F, Thayres dos Santos Lima K, Santos Soares L, José de Andrade C, et al. Flavonoids, anthocyanins, betalains, curcumin, and carotenoids: sources, classification and enhanced stabilization by encapsulation and adsorption. Food Res Int. 2022;153(January):110929.
39. Delgado-Vargas F, Jiménez AR, Paredes-López O. Natural pigments: carotenoids, anthocyanins, and betalains—characteristics, biosynthesis, processing, and stability. Crit Rev Food Sci Nutr. 2000;40(3):173–289.
40. Miguel MG. Betalains in some species of the amaranthaceae family: a review. Antioxidants. 2018;7(4):1–33.
41. Celli GB, Brooks MS. Impact of extraction and processing conditions on betalains and comparison of properties with anthocyanins — a current review. Food Res Int [Internet]. 2017;100:501–9. Available from: https://doi.org/10.1016/j.foodres.2016.08.034.
42. Ngamwonglumlert L, Devahastin S, Chiewchan N. Natural colorants: pigment stability and extraction yield enhancement via utilization of appropriate pretreatment and extraction methods. Crit Rev Food Sci Nutr. 2017;57(15):3243–59.
43. Khan MI. Plant betalains: safety, antioxidant activity, clinical efficacy, and bioavailability. Compr Rev Food Sci Food Saf. 2016;15(2):316–30.
44. Tanaka Y, Sasaki N, Ohmiya A. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J. 2008;54(4):733–49.
45. Otálora MC, Carriazo JG, Iturriaga L, Osorio C, Nazareno MA. Encapsulating betalains from Opuntia ficus-indica fruits by ionic gelation: pigment chemical stability during storage of beads. Food Chem. 2016;202:373–82.
46. Kaimainen M, Laaksonen O, Järvenpää E, Sandell M, Huopalahti R. Consumer acceptance and stability of spray dried betanin in model juices. Food Chem. 2015;187:398–406.
47. Rehman A, Tong Q, Jafari SM, Assadpour E, Shehzad Q, Aadil RM, et al. Carotenoid-loaded nanocarriers: a comprehensive review. Adv Colloid Interface Sci [Internet]. 2020;275:102048. Available from: https://doi.org/10.1016/j.cis.2019.102048.
48. Santos PD d F, Rubio FTV, da Silva MP, Pinho LS, Favaro-Trindade CS. Microencapsulation of carotenoid-rich materials: a review. Food Res Int [Internet]. 2021;147(June):110571. Available from: https://doi.org/10.1016/j.foodres.2021.110571.
49. Jurić S, Jurić M, Król-Kilińska Ż, Vlahoviček-Kahlina K, Vinceković M, Dragović-Uzelac V, et al. Sources, stability, encapsulation and application of natural pigments in foods. Food Rev Int [Internet]. 2020;00(00):1–56. Available from: https://doi.org/10.1080/87559129.2020.1837862.
50. Rodriguez-Concepcion M, Avalos J, Bonet ML, Boronat A, Gomez-Gomez L, Hornero-Mendez D, et al. A global perspective on carotenoids: metabolism, biotechnology, and benefits for nutrition and health. Prog Lipid Res. 2018;70(February):62–93.
51. Manzoor M, Singh J, Gani A, Noor N. Valorization of natural colors as health-promoting bioactive compounds: phytochemical profile, extraction techniques, and pharmacological perspectives. Food Chem [Internet]. 2021;362(May):130141. Available from: https://doi.org/10.1016/j.foodchem.2021.130141.

52. Rodriguez-Amaya DB. Update on natural food pigments - a mini-review on carotenoids, anthocyanins, and betalains. Food Res Int [Internet]. 2019;124(2017):200–5. Available from: https://doi.org/10.1016/j.foodres.2018.05.028.
53. Luzardo-Ocampo I, Ramírez-Jiménez AK, Yañez J, Mojica L, Luna-Vital DA. Technological applications of natural colorants in food systems: a review. Foods. 2021;10(3):1–34.
54. De Mejia EG, Zhang Q, Penta K, Eroglu A, Lila MA. The colors of health: chemistry, bioactivity, and market demand for colorful foods and natural food sources of colorants. Annu Rev Food Sci Technol. 2020;11:145–82.
55. Raddatz-Mota D, Pérez-Flores LJ, Carrari F, Mendoza-Espinoza JA, de León-Sánchez FD, Pinzón-López LL, et al. Achiote (Bixa orellana L.): a natural source of pigment and vitamin E. J Food Sci Technol. 2017;54(6):1729–41.
56. Vilar DDA, Vilar MSDA, Moura TFADLE, Raffin FN, Oliveira MRD, Franco CFDO, et al. Traditional uses, chemical constituents, and biological activities of Bixa Orellana L.: a review. Sci World J. 2014;2014:857292.
57. Shahid-ul-Islam RLJ, Mohammad F. Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications – a review. J Adv Res [Internet]. 2016;7(3):499–514. Available from: https://doi.org/10.1016/j.jare.2015.11.002.
58. EFSA (European Food Safety Authority). The safety of annatto extracts (E 160b) as a food additive. EFSA J. 2016;14(8):e04544.
59. Sharma P, Segat A, Kelly AL, Sheehan JJ. Colorants in cheese manufacture: production, chemistry, interactions, and regulation. Compr Rev Food Sci Food Saf. 2019;19(October):1–23.
60. Rivera-Madrid R, Aguilar-Espinosa M, Cárdenas-Conejo Y, Garza-Caligaris LE. Carotenoid derivates in achiote (Bixa orellana) seeds: synthesis and health promoting properties. Front Plant Sci. 2016;7(September):1–7.
61. EFSA (European Food Safety Authority). Scientific opinion on the re-evaluation of mixed carotenes (E 160a (i)) and beta-carotene (E 160a (ii)) as a food additive. EFSA J. 2012;10(3):1–67.
62. Grune T, Lietz G, Palou A, Ross AC, Stahl W, Tang G, et al. β-carotene is an important vitamin A source for humans. J Nutr. 2010;140(12):2268S–85S.
63. Saini RK, Keum YS. Carotenoid extraction methods: a review of recent developments. Food Chem [Internet]. 2018;240(April 2017):90–103. Available from: https://doi.org/10.1016/j.foodchem.2017.07.099.
64. Rammuni MN, Ariyadasa TU, Nimarshana PHV, Attalage RA. Comparative assessment on the extraction of carotenoids from microalgal sources: astaxanthin from H. pluvialis and β-carotene from D. salina. Food Chem [Internet]. 2019;277(October 2018):128–34. Available from: https://doi.org/10.1016/j.foodchem.2018.10.066.
65. Harvey PJ, Ben-Amotz A. Towards a sustainable Dunaliella salina microalgal biorefinery for 9-cis β-carotene production. Algal Res [Internet]. 2020;50(July):102002. Available from: https://doi.org/10.1016/j.algal.2020.102002.
66. Mercadante AZ, Rodrigues DB, Petry FC, Mariutti LRB. Carotenoid esters in foods – a review and practical directions on analysis and occurrence. Food Res Int [Internet]. 2017;99:830–50. Available from: https://doi.org/10.1016/j.foodres.2016.12.018.
67. Maurya VK, Shakya A, Aggarwal M, Gothandam KM, Bohn T, Pareek S. Fate of β-carotene within loaded delivery systems in food: state of knowledge. Antioxidants. 2021;10(3):1–49.
68. Carvalho GC, de Camargo BAF, de Araújo JTC, Chorilli M. Lycopene: from tomato to its nutraceutical use and its association with nanotechnology. Trends Food Sci Technol. 2021;118(October):447–58.
69. EFSA (European Food Safety Authority). Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food Adopted on 7 March 2008. EFSA J. 2008;721(80):1–29.
70. Shi J, Le Maguer M. Lycopene in tomatoes: chemical and physical properties affected by food processing. Crit Rev Food Sci Nutr. 2000;40:1–42.

71. Chang Y, Jiao Y, Li D-J, Liu X-L, Han H. Glycosylated zein as a novel nanodelivery vehicle for lutein. Food Chem [Internet]. 2022;376(December 2021):131927. Available from: https://doi.org/10.1016/j.foodchem.2021.131927.
72. Gansukh E, Mya KK, Jung M, Keum YS, Kim DH, Saini RK. Lutein derived from marigold (Tagetes erecta) petals triggers ROS generation and activates Bax and caspase-3 mediated apoptosis of human cervical carcinoma (HeLa) cells. Food Chem Toxicol [Internet]. 2019;127(October 2018):11–8. Available from: https://doi.org/10.1016/j.fct.2019.02.037.
73. Steiner BM, McClements DJ, Davidov-Pardo G. Encapsulation systems for lutein: a review. Trends Food Sci Technol. 2018;82(October):71–81.
74. Zeni ALB, Camargo A, Dalmagro AP. Lutein prevents corticosterone-induced depressive-like behavior in mice with the involvement of antioxidant and neuroprotective activities. Pharmacol Biochem Behav. 2019;179:63–72.
75. Kim J, Lee J, Oh JH, Chang HJ, Sohn DK, Kwon O, et al. Dietary lutein plus zeaxanthin intake and DICER1 rs3742330 a > G polymorphism relative to colorectal cancer risk. Sci Rep [Internet]. 2019;9(1):1–8. Available from: https://doi.org/10.1038/s41598-019-39747-5.
76. Toragall V, Srirangam P, Jayapala N, Baskaran V. Lutein encapsulated oleic - linoleic acid nanoemulsion boosts oral bioavailability of the eye protective carotenoid lutein in rat model. Mater Today Commun [Internet]. 2021;28(May):102522. Available from: https://doi.org/10.1016/j.mtcomm.2021.102522.
77. Ding Z, Tao T, Yin X, Prakash S, Wang X, Zhao Y, et al. Improved encapsulation efficiency and storage stability of spray dried microencapsulated lutein with carbohydrates combinations as encapsulating material: microencapsulated spray-dried lutein. Lebensm Wiss Technol. 2020;124:109139.
78. Hao J, Xu J, Zhang W, Li X, Liang D, Xu D, et al. The improvement of the physicochemical properties and bioaccessibility of lutein microparticles by electrostatic complexation. Food Hydrocoll [Internet]. 2022;125(October 2021):107381. Available from: https://doi.org/10.1016/j.foodhyd.2021.107381.
79. Šeregelj V, Ćetković G, Čanadanović-Brunet J, Šaponjac VT, Vulić J, Lević S, et al. Encapsulation of carrot waste extract by freeze and spray drying techniques: an optimization study. Lebensm Wiss Technol. 2021;138(August 2020):1–10.
80. Chang R, Yang J, Ge S, Zhao M, Liang C, Xiong L, et al. Synthesis and self-assembly of octenyl succinic anhydride modified short glucan chains based amphiphilic biopolymer: micelles, ultrasmall micelles, vesicles, and lutein encapsulation/release. Food Hydrocoll [Internet]. 2017;67:14–26. Available from: https://doi.org/10.1016/j.foodhyd.2016.12.023.
81. Zhao L, Temelli F, Curtis JM, Chen L. Encapsulation of lutein in liposomes using supercritical carbon dioxide. Food Res Int [Internet]. 2017;100:168–79. Available from: https://doi.org/10.1016/j.foodres.2017.06.055.
82. Qi X, Xu D, Zhu J, Wang S, Peng J, Gao W, et al. Interaction of ovalbumin with lutein dipalmitate and their effects on the color stability of marigold lutein esters extracts. Food Chem [Internet]. 2022;372(11):131211. Available from: https://doi.org/10.1016/j.foodchem.2021.131211.
83. EFSA (European Food Safety Authority). Scientific opinion on the re-evaluation of paprika extract (E 160c) as a food additive. EFSA J. 2015;13(12):1–52.
84. Arimboor R, Natarajan RB, Menon KR, Chandrasekhar LP, Moorkoth V. Red pepper (Capsicum annuum) carotenoids as a source of natural food colors: analysis and stability—a review. J Food Sci Technol. 2015;52(3):1258–71.
85. Rowe A. Global cochineal production: scale, welfare concerns, and potential interventions. OSF Preprints. 2020. p. 1–15.
86. Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, Galtier P, et al. EFSA ANS panel (EFSA panel on food additives and nutrient sources added to food). Sci Opin re-evaluation cochineal, carminic acid, carmines as a food. Addit Eur Food Saf Auth J. 2015;13:4288.

87. Zakariya SM, Furkan M, Zaman M, Chandel TI, Ali SM, Uversky VN, et al. An in-vitro elucidation of inhibitory potential of carminic acid: possible therapeutic approach for neurodegenerative diseases. J Mol Liq. 2020;303:112692.
88. Rader Bowers LM, Schmidtke Sobeck SJ. Impact of medium and ambient environment on the photodegradation of carmine in solution and paints. Dyes Pigments. 2016;127:18–24.
89. Ghidouche S, Rey B, Michel M, Galaffu N. A rapid tool for the stability assessment of natural food colours. Food Chem. 2013;139(1–4):978–85.
90. Campana MG, Robles García NM, Tuross N. America's red gold: multiple lineages of cultivated cochineal in Mexico. Ecol Evol. 2015;5(3):607–17.
91. Cooksey CJ. The red insect dyes: carminic, kermesic and laccaic acids and their derivatives. Biotech Histochem. 2019;94(2):100–7.
92. Yilmaz UT, Ergun F, Yilmaz H. Determination of the food dye carmine in milk and candy products by differential pulse polarography. J Food Drug Anal. 2014;22(3):329–35.
93. Sharma M, Usmani Z, Gupta VK, Bhat R. Valorization of fruits and vegetable wastes and by-products to produce natural pigments. Crit Rev Biotechnol [Internet]. 2021;41(4):535–63. Available from: https://doi.org/10.1080/07388551.2021.1873240.
94. Su S, Zhou Y, Qin JG, Yao W, Ma Z. Optimization of the method for chlorophyll extraction in aquatic plants. J Freshw Ecol. 2010;25(4):531–8.
95. Lee J, Kwak M, Chang YK, Kim D. Green solvent-based extraction of chlorophyll a from Nannochloropsis sp. using 2,3-butanediol. Sep Purif Technol [Internet]. 2021;276(July):119248. Available from: https://doi.org/10.1016/j.seppur.2021.119248.
96. Morcelli A, Cassel E, Vargas R, Rech R, Marcílio N. Supercritical fluid (CO2+ethanol) extraction of chlorophylls and carotenoids from Chlorella sorokiniana: COSMO-SAC assisted prediction of properties and experimental approach. J CO2 Util [Internet]. 2021;51(May):101649. Available from: https://doi.org/10.1016/j.jcou.2021.101649.
97. Tavakoli S, Hong H, Wang K, Yang Q, Gahruie HH, Zhuang S, et al. Ultrasonic-assisted food-grade solvent extraction of high-value added compounds from microalgae Spirulina platensis and evaluation of their antioxidant and antibacterial properties. Algal Res [Internet]. 2021;60(September):102493. Available from: https://doi.org/10.1016/j.algal.2021.102493.
98. Vaz BMC, Martins M, de Souza Mesquita LM, Neves MC, Fernandes APM, Pinto DCGA, et al. Using aqueous solutions of ionic liquids as chlorophyll eluents in solid-phase extraction processes. Chem Eng J. 2022;428(July 2021):131073.
99. EFSA (European Food Safety Authority). Scientific opinion on re-evaluation of copper complexes of chlorophylls (E 141(i)) and chlorophyllins (E 141(ii)) as food additives. EFSA J. 2015;13(6):1–60.
100. Wang F, Terazono Y, Liu J, Fefer M, Pelton RH. Adsorption of aqueous copper chlorophyllin mixtures on model surfaces. Colloids Surf A Physicochem Eng Asp [Internet]. 2020;592(December 2019):124578. Available from: https://doi.org/10.1016/j.colsurfa.2020.124578.
101. Gandul-Rojas B, Roca M, Gallardo-Guerrero L. Detection of the color adulteration of green table olives with copper chlorophyllin complexes (E-141ii colorant). LWT – Food Sci Technol [Internet]. 2012;46(1):311–8. Available from: https://doi.org/10.1016/j.lwt.2011.09.012.
102. Zielinski AAF, Sanchez-Camargo A d P, Benvenutti L, Ferro DM, Dias JL, Ferreira SRS. High-pressure fluid technologies: recent approaches to the production of natural pigments for food and pharmaceutical applications. Trends Food Sci Technol. 2021;118(October):850–69.
103. Almeida HHS, Barros L, Barreira JCM, Calhelha RC, Heleno SA, Sayer C, et al. Bioactive evaluation and application of different formulations of the natural colorant curcumin (E100) in a hydrophilic matrix (yogurt). Food Chem [Internet]. 2018;261(April):224–32. Available from: https://doi.org/10.1016/j.foodchem.2018.04.056.
104. Neves MIL, Desobry-Banon S, Perrone IT, Desobry S, Petit J. Encapsulation of curcumin in milk powders by spray-drying: physicochemistry, rehydration properties, and stability during storage. Powder Technol. 2019;345:601–7.

105. Rao PJ, Khanum H. A green chemistry approach for nanoencapsulation of bioactive compound – Curcumin. LWT – Food Sci Technol [Internet]. 2016;65:695–702. Available from: https://doi.org/10.1016/j.lwt.2015.08.070.
106. Tai K, Rappolt M, Mao L, Gao Y, Yuan F. Stability and release performance of curcumin-loaded liposomes with varying content of hydrogenated phospholipids. Food Chem [Internet]. 2020;326(May):126973. Available from: https://doi.org/10.1016/j.foodchem.2020.126973.
107. Sigurdson GT, Tang P, Giusti MM. Natural colorants: food colorants from natural sources. Annu Rev Food Sci Technol. 2017;8:261–80.
108. Pez Jaeschke D, Rocha Teixeira I, Damasceno Ferreira Marczak L, Domeneghini Mercali G. Phycocyanin from Spirulina: a review of extraction methods and stability. Food Res Int. 2021;143(October 2020):110314.
109. Galetovic A, Seura F, Gallardo V, Graves R, Cortés J, Valdivia C, et al. Use of phycobiliproteins from Atacama cyanobacteria as food colorants in a dairy beverage prototype. Foods. 2020;9(2):1–13.
110. Bord C, Guerinon D, Lebecque A. Heated or raw blue cheeses: what are the drivers influencing consumer preferences? Int J Food Sci Technol. 2017;52(9):1959–70.
111. Carocho M, Morales P, Ferreira ICFR. Natural food additives: quo vadis? Trends Food Sci Technol. 2015;45(2):284–95.
112. Martins N, Roriz CL, Morales P, Barros L, Ferreira ICFR. Food colorants: challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. Trends Food Sci Technol. 2016;52:1–15.
113. Backes E, Leichtweis MG, Pereira C, Carocho M, Barreira JCM, Kamal Genena A, et al. Ficus carica L. and Prunus spinosa L. extracts as new anthocyanin-based food colorants: a thorough study in confectionery products. Food Chem. 2020;333(February):127457.
114. Vázquez-Carrillo MG, Aparicio-Eusebio LA, Salinas-Moreno Y, Buendía-Gonzalez MO, Santiago-Ramos D. Nutraceutical, physicochemical, and sensory properties of blue corn polvorones, a traditional flour-based confectionery. Plant Foods Hum Nutr. 2018;73(4):321–7.
115. Baldin J, Michelin E, Polizer Y, Rodrigues I, Godoy S, Fregonesi R, et al. Microencapsulated jabuticaba (Myrciaria cauliflora) extract added to fresh sausage as natural dye with antioxidant and antimicrobial activity. Meat Sci. 2016;118:15–21.
116. Yao X, Liu J, Hu H, Yun D, Liu J. Development and comparison of different polysaccharide/PVA-based active/intelligent packaging films containing red pitaya betacyanins. Food Hydrocoll. 2022;124(PB):107305.
117. Etxabide A, Maté JI, Kilmartin PA. Effect of curcumin, betanin and anthocyanin containing colourants addition on gelatin films properties for intelligent films development. Food Hydrocoll. 2021;115(January):106593.
118. Carvalho AS d, Rezende SC d, Caleja C, Pereira E, Barros L, Fernandes I, et al. β-Carotene colouring systems based on solid lipid particles produced by hot melt dispersion. Food Control. 2021;129(May):108262.
119. Landim Parente GD, Nunes de Melo BD, Albuquerque de Souza J, da Conceição MM, Ubbink J, Mattos Braga AL. Fortification of traditional tapioca "pancakes" from the Brazilian northeast with microencapsulated carrot carotenoid. Lwt. 2021;152(May):112301.
120. Ordóñez-Santos LE, Esparza-Estrada J, Vanegas-Mahecha P. Ultrasound-assisted extraction of total carotenoids from mandarin epicarp and application as natural colorant in bakery products. Lwt. 2021;139(June 2020):110598.
121. Tupuna-Yerovi DS, Paese K, Flôres SH, Guterres SS, Rios A. Addition of norbixin microcapsules obtained by spray drying in an isotonic tangerine soft drink as a natural dye. J Food Sci Technol. 2020;57(3):1021–31.
122. de BARROS RF, Torres FR, da SILVA PHF, Stringheta PC, Pereira JPF, de PAULA JCJ, et al. Lutein as a functional ingredient in sheep milk yogurt: development, characterization and extraction recovery. Food Sci Technol. 2020;40(December):683–90.
123. Cerezal Mezquita P, Morales J, Palma J, Ruiz MDC, Jáuregui M. Stability of Lutein Obtained from Muriellopsis sp biomass and used as a natural colorant and antioxidant in a mayonnaise-

like dressing sauce. CYTA – J Food [Internet]. 2019;17(1):517–26. Available from: https://doi.org/10.1080/19476337.2019.1609091.
124. Mehta D, Prasad P, Sangwan RS, Yadav SK. Tomato processing byproduct valorization in bread and muffin: improvement in physicochemical properties and shelf life stability. J Food Sci Technol [Internet]. 2018;55(7):2560–8. Available from: https://doi.org/10.1007/s13197-018-3176-0.
125. Marchetti N, Bonetti G, Brandolini V, Cavazzini A, Maietti A, Meca G, et al. Stinging nettle (Urtica dioica L.) as a functional food additive in egg pasta: enrichment and bioaccessibility of Lutein and β-carotene. J Funct Foods [Internet]. 2018;47(March):547–53. Available from: https://doi.org/10.1016/j.jff.2018.05.062.
126. Teixeira VMC, da Silva RFG, Gonçalves OH, Pereira C, Barros L, Ferreira ICFR, et al. Chemometric approaches to evaluate the substitution of synthetic food dyes by natural compounds: the case of nanoencapsulated curcumin, spirulina, and hibiscus extracts. Lwt. 2022;154:112786.
127. Tosati JV, de Oliveira EF, Oliveira JV, Nitin N, Monteiro AR. Light-activated antimicrobial activity of turmeric residue edible coatings against cross-contamination of Listeria innocua on sausages. Food Control. 2018;84:177–85.
128. Batista AP, Niccolai A, Fradinho P, Fragoso S, Bursic I, Rodolfi L, et al. Microalgae biomass as an alternative ingredient in cookies: sensory, physical and chemical properties, antioxidant activity and in vitro digestibility. Algal Res. 2017;26(June):161–71.
129. Tereucan G, Ercoli S, Cornejo P, Winterhalter P, Contreras B, Ruiz A. Stability of antioxidant compounds and activities of a natural dye from coloured-flesh potatoes in dairy foods. Lebensm Wiss Technol. 2021;144(March):111252.
130. Swer TL, Chauhan K, Mukhim C, Bashir K, Kumar A. Application of anthocyanins extracted from Sohiong (Prunus nepalensis L.) in food processing. Lebensm Wiss Technol. 2019;114(June):108360.
131. Byamukama R, Andima M, Mbabazi A, Kiremire BT. Anthocyanins from mulberry (Morus rubra) fruits as potential natural colour additives in yoghurt. African J Pure Appl Chem. 2014;8(12):182–90.
132. Pires TCSP, Dias MI, Barros L, Barreira JCM, Santos-Buelga C, Ferreira ICFR. Incorporation of natural colorants obtained from edible flowers in yogurts. Lebensm Wiss Technol. 2018;97(July):668–75.
133. Sampaio SL, Lonchamp J, Dias MI, Liddle C, Petropoulos SA, Glamočlija J, et al. Anthocyanin-rich extracts from purple and red potatoes as natural colourants: bioactive properties, application in a soft drink formulation and sensory analysis. Food Chem. 2021;342(October 2020):128526.
134. Montibeller MJ, de Lima MP, Tupuna-Yerovi DS, Rios A d O, Manfroi V. Stability assessment of anthocyanins obtained from skin grape applied in kefir and carbonated water as a natural colorant. J Food Process Preserv. 2018;42(8):1–10.
135. Albuquerque BR, Pinela J, Barros L, Oliveira MBPP, Ferreira ICFR. Anthocyanin-rich extract of jabuticaba epicarp as a natural colorant: optimization of heat- and ultrasound-assisted extractions and application in a bakery product. Food Chem. 2020;316:126364.
136. Zheng Y, Li X, Huang Y, Li H, Chen L, Liu X. Two colorimetric films based on chitin whiskers and sodium alginate/gelatin incorporated with anthocyanins for monitoring food freshness. Food Hydrocoll. 2022;127(January):107517.
137. Sani MA, Tavassoli M, Hamishehkar H, McClements DJ. Carbohydrate-based films containing pH-sensitive red barberry anthocyanins: application as biodegradable smart food packaging materials. Carbohydr Polym [Internet]. 2021;255(December 2020):117488. Available from: https://doi.org/10.1016/j.carbpol.2020.117488.
138. Wu LT, Tsai IL, Ho YC, Hang YH, Lin C, Tsai ML, et al. Active and intelligent gellan gum-based packaging films for controlling anthocyanins release and monitoring food freshness. Carbohydr Polym. 2021;254(June 2020):117410.

139. Boonsiriwit A, Lee M, Kim M, Inthamat P, Siripatrawan U, Lee YS. Hydroxypropyl methylcellulose/microcrystalline cellulose biocomposite film incorporated with butterfly pea anthocyanin as a sustainable pH-responsive indicator for intelligent food-packaging applications. Food Biosci. 2021;44(PA):101392.
140. Wen Y, Liu J, Jiang L, Zhu Z, He S, He S, et al. Development of intelligent/active food packaging film based on TEMPO-oxidized bacterial cellulose containing thymol and anthocyanin-rich purple potato extract for shelf life extension of shrimp. Food Packag Shelf Life. 2021;29(June):100709.
141. He Y, Li B, Du J, Cao S, Liu M, Li X, et al. Development of pH-responsive absorbent pad based on polyvinyl alcohol/agarose/anthocyanins for meat packaging and freshness indication. Int J Biol Macromol. 2022;201(January):203–15.
142. Alizadeh Sani M, Tavassoli M, Salim SA, Azizi-lalabadi M, McClements DJ. Development of green halochromic smart and active packaging materials: TiO2 nanoparticle- and anthocyanin-loaded gelatin/κ-carrageenan films. Food Hydrocoll. 2022;124(November 2021):107324.
143. Otálora MC, de Jesús BH, Perilla JE, Osorio C, Nazareno MA. Encapsulated betalains (Opuntia ficus-indica) as natural colorants. Case study: gummy candies. Lwt [Internet]. 2019;103(December 2018):222–7. Available from: https://doi.org/10.1016/j.lwt.2018.12.074.
144. Moghaddas Kia E, Ghaderzadeh S, Mojaddar Langroodi A, Ghasempour Z, Ehsani A. Red beet extract usage in gelatin/gellan based gummy candy formulation introducing Salix aegyptiaca distillate as a flavouring agent. J Food Sci Technol. 2020;57(9):3355–62.
145. Kharrat N, Salem H, Mrabet A, Aloui F, Triki S, Fendri A, et al. Synergistic effect of polysaccharides, betalain pigment and phenolic compounds of red prickly pear (Opuntia stricta) in the stabilization of salami. Int J Biol Macromol. 2018;111:561–8.
146. Yang W, Kaimainen M, Järvenpää E, Sandell M, Huopalahti R, Yang B, et al. Red beet (Beta vulgaris) betalains and grape (Vitis vinifera) anthocyanins as colorants in white currant juice – effect of storage on degradation kinetics, color stability and sensory properties. Food Chem. 2021;348(January):128995.
147. Yao X, Hu H, Qin Y, Liu J. Development of antioxidant, antimicrobial and ammonia-sensitive films based on quaternary ammonium chitosan, polyvinyl alcohol and betalains-rich cactus pears (Opuntia ficus-indica) extract. Food Hydrocoll [Internet]. 2020;106(February):105896. Available from: https://doi.org/10.1016/j.foodhyd.2020.105896.
148. Qin Y, Liu Y, Zhang X, Liu J. Development of active and intelligent packaging by incorporating betalains from red pitaya (Hylocereus polyrhizus) peel into starch/polyvinyl alcohol films. Food Hydrocoll. 2020;100(August 2019):105410.
149. Lobo FATF, Silva V, Domingues J, Rodrigues S, Costa V, Falcão D, et al. Inclusion complexes of yellow bell pepper pigments with β-cyclodextrin: preparation, characterisation and application as food natural colorant. J Sci Food Agric. 2018;98(7):2665–71.
150. Sobral D, Bueno Costa RG, Machado GM, Jacinto de Paula JC, Martins Teodoro VA, Nunes NM, et al. Can lutein replace annatto in the manufacture of Prato cheese? LWT – Food Sci Technol. 2016;68:349–55.
151. Liu CX, Wang C, Liu JX, Ren DX. Effect of feed lutein supplementation on mozzarella cheese quality and lutein stability. Int Dairy J [Internet]. 2018;83:28–33. Available from: https://doi.org/10.1016/j.idairyj.2018.03.008.
152. Domingos LD, Xavier AAO, Mercadante AZ, Petenate AJ, Jorge RA, Viotto WH. Oxidative stability of yogurt with added lutein dye. J Dairy Sci [Internet]. 2014;97(2):616–23. Available from: https://doi.org/10.3168/jds.2013-6971.
153. Pöhnl H. Applications of different curing approaches and natural colorants in meat products. In: Handbook on natural pigments in food and beverages: industrial applications for improving food color. Elsevier Ltd; 2016. p. 209–25.
154. Ongaratto GC, Oro G, Kalschne DL, Trindade Cursino AC, Canan C. Cochineal carmine adsorbed on layered zinc hydroxide salt applied on mortadella to improve color stability. Curr Res Food Sci. 2021;4(July):758–64.

155. Fradinho P, Niccolai A, Soares R, Rodolfi L, Biondi N, Tredici MR, et al. Effect of Arthrospira platensis (spirulina) incorporation on the rheological and bioactive properties of gluten-free fresh pasta. Algal Res. 2020;45(December 2019):101743.
156. Bazarnova J, Nilova L, Trukhina E, Bernavskaya M, Smyatskaya Y, Aktar T. Use of microalgae biomass for fortification of food products from grain. Foods. 2021;10(12):3018.
157. Yasuda M, Tabata M. Effect of emulsifiers on the discoloration of chlorophyll and their potential for use in green beverages. J Food Sci. 2021;86(7):3033–45.
158. Chen H z, Zhang M, Bhandari B, Guo Z. Applicability of a colorimetric indicator label for monitoring freshness of fresh-cut green bell pepper. Postharvest Biol Technol [Internet]. 2018;140(November 2017):85–92. Available from: https://doi.org/10.1016/j.postharvbio.2018.02.011.
159. Saraiva BB, Rodrigues BM, da Silva Junior RC, Scapim MR d S, Lancheros CAC, Nakamura CV, et al. Photodynamic inactivation of Pseudomonas fluorescens in Minas Frescal cheese using curcumin as a photosensitizer. Lwt. 2021;151(July):112143.
160. KOOP BL, Cargnin MA, Fidler F, Ribeiro DHB, de Campos CEM, Soares LS, et al. Vacuum curcumin infusion in cooked oysters (Crassostrea gigas) to increase their shelf life. J Food Process Eng. 2019;42(6):1–11.
161. Song HY, McClements DJ. Nano-enabled-fortification of salad dressings with curcumin: impact of nanoemulsion-based delivery systems on physicochemical properties. Lwt [Internet]. 2021;145(March):111299. Available from: https://doi.org/10.1016/j.lwt.2021.111299.
162. Yildiz E, Sumnu G, Kahyaoglu LN. Monitoring freshness of chicken breast by using natural halochromic curcumin loaded chitosan/PEO nanofibers as an intelligent package. Int J Biol Macromol [Internet]. 2021;170:437–46. Available from: https://doi.org/10.1016/j.ijbiomac.2020.12.160.
163. Tosati JV, Messias VC, Carvalho PIN, Rodrigues Pollonio MA, Meireles MAA, Monteiro AR. Antimicrobial effect of edible coating blend based on turmeric starch residue and gelatin applied onto fresh frankfurter sausage. Food Bioprocess Technol. 2017;10(12):2165–75.

Chapter 5
Natural Sweeteners

Lina Maria Rayo-Mendez and Jaiber Humberto Rodriguez-Llanos

5.1 Introduction

Natural sweeteners are substitutes for sucrose also known as "table sugar", commonly obtained from sugar cane (*Saccharum officinarum*) [1] and sugar beet (*Beta vulgaris*) [2], also substitutes for honey and maple syrup. These sweeteners are food additives (FAs) that provide or mimic a sweet flavor similar to sugar (sucrose) but with less caloric energy, then their impact on the diet is remarkably considered. Since the eighteenth century, the use of sugar in the form of sucrose extracted from sugar cane was widespread in the food industry and population [3, 4]. Over the years, metabolic disorders, obesity, and diabetes mellitus were increasing worldwide, becoming a health problem, since those conditions can trigger other diseases, thus, a non-caloric, non-nutritive alternative was necessary to sweeten beverages and foods. The first sweetener discovered was saccharine in 1879, a synthetic sweetener developed apparently as a suitable option for diabetic people [3]. Although sweeteners play an essential role in health and being safe for human diet, they are also important for the food industry since sweeteners are FAs that can provide specific characteristics to food products [5].

Water solubility, low rate of dissolution, temperature stability, colorless, odorless, length shelf life, non-toxic, non-after taste, low-cost and availability to produce it, among others [1, 6] are characteristics should be remarkably considered to choose a determine sweetener to food applications, beyond to sweeten foods, sweeteners are additives which also can provide properties of texture, moisture, bulk, flavor,

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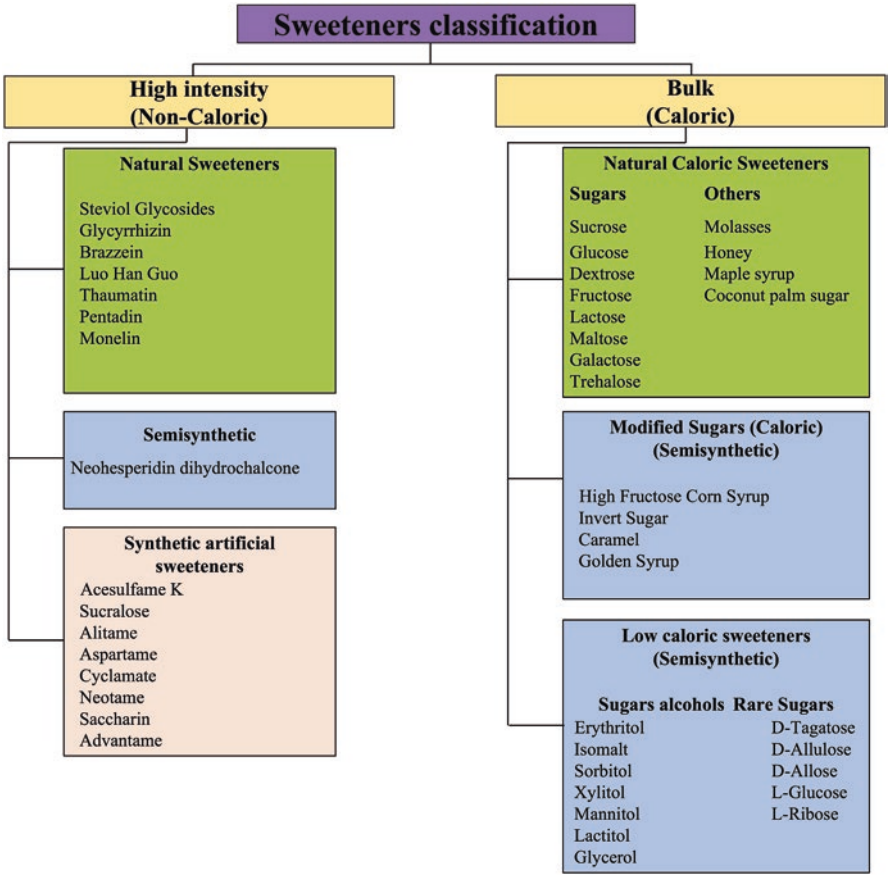

Fig. 5.1 Types of sweeteners and their classification according to calorie intake. (Adapted from García-Almeida et al. [7])

and color. In general, sweeteners can be categorized along two lines as high intensity (Non-caloric) or bulk (Caloric) (Fig. 5.1). Consequently, those two categories can be further divided into natural, semisynthetic, and artificial sweeteners (synthetic), depending on origin and production [8]. Categories as non-nutritive and nutritive, high, and low potency are usually used in the food industry, but still, the primary vantage of sweeteners is related to their low calories.

Natural (caloric) sweeteners are understood as minimally processed products extracted from plants or natural resources, these include sugars (sucrose, fructose, glucose, maltose, lactose, maltose, galactose, among others), honey, maple syrup, molasses, and coconut palm sugar, nonetheless with a high caloric level. In contrast, natural sweeteners (Non-caloric) have been also extracted from naturals sources (plants) or have undergone physicochemical processes, but all low calories, in this group may include Steviol glycosides (SGs), Glycyrrhizin, Thaumatin, Pentadin, Brazzein and Monellin [9]. Next are artificial sweeteners (synthetic), made strictly

from chemical substances through different reactions, which have high intensity sweetness power, such as acesulfame K, aspartame, alitame, cyclamate, neotame, saccharin, and sucralose. All of them have been applied in several foods and beverages such as desserts, canned foods, dairy products, baked goods, carbonated beverages, powdered drink mixes, soups, among others. However, artificial sweeteners consumption is regulated by the U.S. Food and Drug Administration by means of the acceptable daily intake (ADI) value, in view of some controversial safety health effects and instability of these sweeteners when applied in foods [10]. Low caloric sweeteners (semisynthetic) include sugars alcohols (erythritol, maltitol, lactitol, mannitol, glycerol, xylitol, isomalt) [11] and rare sugars (D-Tagatose, D-Allulose, D-Allose, L-Glucose, L-Ribose) [12], they are known also as semi-naturals because can be often found in foods or plants, however, are industrially treated through enzymes, yeasts or fungi to produce them [13]. As well as natural sweeteners can be applied in different preparations such as beverages, yogurts, cookies, cakes, and even in the cosmetic industry such as toothpaste, dental rinses, and so on [14]. According to Scoot et al. [15], people are becoming more conscious about their quality of life in terms of controlling weight and keeping it at acceptable levels. Thus, consumers in recent decades have focused on making choices for food products that do not directly affect health. On the other hand, the food industry has designed the development of lines of research into healthier foods for this market niche.

5.2 Main Natural Sweeteners: Properties and Applications

5.2.1 Erythritol

Erythritol is a natural sweetener found in fruits like grapes (0–42 mg/kg), melons (22–47 mg/kg), pears (0–40 mg/kg), mushrooms, seaweeds, wines (130–300 mg/l), beers, soy sauce (910 mg/l), sake (1550 mg/l), miso bean paste (1310 mg/l) [16, 17]. It was discovered in 1948 by the Scottish chemist John Stenhouse, isolated in 1952, and finally introduced to the Japanese market in 1990 as a sugar substitute for soft drinks, chewing gum, jams, and candies, and nowadays it is used as a growing ingredient [18–21]. For large-scale industrial production, erythritol can be obtained through several chemical methods, also extraction, however, fermentation is a more cost-effective process to produce it [17, 19]. Nowadays, glucose fermentation with yeast-like fungi such as Moniliella pollinis, Trichosporonoides megachiliensis, and Yarrowia lipolytica are commonly employed due to their high production. After the separation and purification process, erythritol results as a white-crystalline granular substance apparently similar to table sugar with a percent relative sweetness between 60% and 70% when compared to sucrose [17], but with a glycemic index of 0 [5], being its most valuable property.

It belongs to the carbohydrate family known as a sugar alcohol, or polyol or polyhydric alcohol because of its hydroxyl groups (HO^- radical) on the formula (Fig. 5.2). The polyols family is a class of low molecular weight compounds with a

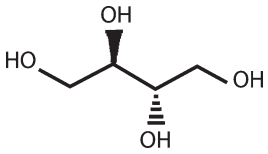

Fig. 5.2 Chemical structure of erythritol

general formula of (CHOH)n H_2 where n = 4–6. Erythritol is a 4-carbon sugar alcohol, its formula is $C_4H_{10}O_4$, whose properties are: molar weight of 122.120 g/mol, density of 1.45 g/cm^3, melting point at 126 °C, a boiling point between 329 and 331 °C, solubility of 38% at 25 °C (61 g/100 g water), heat of solution of −43.9 cal/g [17, 21–23]. In spite of the name sugar alcohol, erythritol is a noncaloric organic, non-glycemic sweetener, then is safe for diabetic patients because there is no influence on blood insulin levels due to its chemical structure [17] and is secreted by urine [22].

Erythritol was approved as safe for consumption by FDA (Food and drug Administration) in the United States and also in the European Union (EU) under the code of E968 [18, 24, 25]. Because of its small molecular size, erythritol has a high digestive tolerance being absorbed into the small intestine and is no fermented in the large intestine as other polyols do, exhibiting a vantage among other sweeteners to use in food formulation. Although, doses should be regulated, due to excess larger than 50 g of intake can lead to borborygmus and nausea [26], according to Oku and Okazaki [27], the highest safe dose of erythritol is 0.80 g/kg BW for females and 0.66 g/kg body weight (BW) for males to avoid laxative effect on the body. Tetzloff et al. [28] observed that even daily doses of 1 g/kg BW were safe for humans without gastrointestinal effects and urinary electrolytes excretion was not affected.

Erythritol has been extensively used in foods due to its sweetness profile (60–70%) and texture (small crystals), similar to sucrose. The most common is the table-top use, but also it is a flavor enhancer in foods because of its large cooling effect when dissolved in water, an endothermic reaction caused for its high negative heat of solution of −43.9 cal/g. Although this is a normal characteristic of polyols, erythritol has the highest value when compared to the others and even sucrose. Perko et al. [23] studied the drop in temperature (cooling effect) of different polyols, dissolving 30 g of each sweetener in 100 g of water; authors observed that sorbitol, xylitol and erythritol temperature dropped from 37 °C to 31, 29, and 27 °C, respectively, and for maltitol, isomalt, and sucrose, temperature decrease only close to 35 °C for 13 s of time. The cooling effect might be potentially an advantage in enhancing consumer enjoyment of such mint-flavored products. Therefore, erythritol is commonly combined with a mint flavor creating a feeling of freshness on chewing gums, hard candies, frostings, ice creams and low-calorie beverages [6, 29–31]. Also, one of the advantages to used erythritol in food formulations is the no contribution to the formation of tooth decay caused by dental plaque turning sugars into acid. Some of erythritol properties are summarized in Table 5.1. Erythritol is stable to acid and alkaline pH conditions, also to high temperatures even until 180 °C without decomposition. It is a bulk sweetener providing volume which makes it a good ingredient

Table 5.1 Applications of erythritol in food products

Properties	Industrial use	Product	References
Sweetener, flavor enhancer and cooling sensation	Food	Frostings, chewing gum, hard candy, glazed goods, and chocolate	[6, 23, 29, 32]
Sweetener and texture	Bakery products	Cookies, muffins, and cakes	[33–36]

to use in baked products such as cookies [33, 34], cakes [18, 35] and muffins [36], with good hedonic acceptance from consumers when compared to sugar products.

Akesowan [35] evaluated chiffon cakes containing 0, 25, 50, 75 and 100% of erythritol as a blend with sucralose as sugar (sucrose) replacer. Cakes made with 50% of erythritol-sucralose had better scores an acceptable sensory quality similar when compared with control cake. Cake moistness and water activity increased as the level of erythritol-sucralose rise, while tenderness decreased. At 100% level, cake batter had a lower ability to retain air resulting in a compacted cake. These results were attributed to lower sweetener solubility in water compared to sucrose, leaving more available water to the process. But also, because sugar (sucrose) plays a role in wheat flour, used to produce cakes, delaying starch gelatinization affecting its temperature. Sucrose binds the amylose and amylopectin chains of starch in the amorphous zones of the granules, stabilizing them. These bridges increase energy requirements, which results in higher gelatinization temperatures [37]. Sweeteners are no able to create these bridges then sometimes it is necessary to use stabilizers such as inulin (a polysaccharide) and soluble fiber, mainly extracted from chicory root with a slightly sweet flavor with zero glycemic index, non-caloric and safe for diabetic people [20], but with properties of high swelling, then inulin bonds with water and can be used as a thickening agent.

Laguna et al. [34] produced cookies with 25% and 50% of erythritol and inulin as a sucrose replacement, obtaining a suitable dough from a processing point of view, and cookies color, but cookies with 50% erythritol were scored negatively for all sensory attributes being texture main. Cookies with 25% of erythritol obtained better scores from consumers' acceptance also were harder than cookies made with inulin which were softer.

Erythritol used in chewing gum provides high flexibility and a soft texture, these properties increase chewing gum shelf life avoiding dryness and hard gums texture, undesirable characteristics for consumers [23].

5.2.2 Other Polyols

Other polyols are recognized as sugar substitutes, some examples are glycerol, xylitol, sorbitol, and mannitol which are derived from monosaccharides, whereas maltitol, isomalt, and lactitol are disaccharide derivatives [1]. Although some are nearly 50% as sweet as sucrose and their glycemic index is lower than sucrose, their consumption is limited once those polyols may cause gastrointestinal discomfort to

human body, then their amount intake is regulated. Maximum bolus doses not causing laxation for sorbitol are 0.17 (males) and 0.80 (females) g/kg body weight, whereas maltitol, isomalt, and xylitol is 0.3 g/kg body weight (both males and female), and for erythritol is 0.66 and 0.80 g/kg body weight for males and females, respectively [23]. The industrial production of these polyols mostly involves the chemical hydrogenation of sugars, but low-cost alternatives such as biotechnological production have been considered during the past years due to the high market demand for low-calorie sweeteners by fermentation through microbial routes [38]. The chemical and physiological characteristics of polyols are summarized in Table 5.2.

Besides erythritol, polyols also have a corresponding number from the European Union such as (E420) sorbitol, (E421) mannitol, (E953) isomalt, (E965) maltitol, (E966) lactitol, xylitol (E967) and erythritol (E968) [40]. Some polyols have been studied as sweeteners for chewing gum production because of their humectant, plasticizing and cooling properties, but also because sugar-free chewing gum has proven to decrease caries incidence, these include xylitol, sorbitol [41] and erythritol [21]. Those sweeteners have gained interest because they are not metabolized by most oral bacteria, then they do not produce acid and the saliva pH does not decrease, a common factor after eating, in contrast, they increase or stimulate saliva production, a positive factor to prevent plaque and caries [41–43]. Jeon et al. [44] observed xylitol affect negatively texture quality on moisture content and hardness of hard candy used in their formulation, because its high hygroscopicity, however blending isomalt, maltitol, and xylitol in different percentages as 90.21%, 8.63%, and 1.16% respectively produced high acceptability from consumers, in hard candies formulated without sugar.

Erythritol is often used in chewing gum coatings, however rough surfaces are obtained due to the fast crystallization of this sweetener, consequently, sorbitol, maltitol, and xylitol represent an alternative to avoid this problem. Analysis of crunchiness and stability against moisture parameters, for chewing gum coatings using sorbitol, xylitol, isomalt, maltitol, and mixtures at a ratio of 40:60 of erythritol/sorbitol and erythritol/maltitol were evaluated (Fig. 5.3). Chewing gum coating with xylitol showed similar results on all parameters, although the erythritol/sorbitol 40:60 represented a suitable alternative to produce a chewing gum low calorie and with consumer acceptance [23]. These findings suggest that polyols combinations may help the desirable characteristics to develop a product.

5.2.3 Tagatose

D-tagatose is a hexose monosaccharide classified as a “rare sugar” according to the ISRS (International Society of Rare Sugars) because minimal quantities are available in nature. In rare sugars group are also found low caloric monosaccharides as the L-glucose a L isomer of glucose synthesized artificially in a laboratory, the D-allulose and L-ribose, among others [12]. Lately, this natural sweetener has

Table 5.2 Chemical and physiological characteristics of sweeteners: chemical formula, systematic (IUPAC) name, percent (%) relative sweetness compared to sucrose[a], glycemic index[b], caloric value (kcal/g), solubility at 25 °C (g/100 ml), heat of solution (cal/g), source, taste, and food application

Sweetener	Chemical formula[a]	Systematic (IUPAC) name[a]	% Relative sweetness vs. sucrose[a]	Glycemic index[b]	Caloric value (kcal/g)[c]	Solubility at 25 °C (g/100 ml)[c]	Heat of solution (cal/g)[c]	Source[d]	Taste[e]	Applications[e]
Sucrose	$C_{12}H_{22}O_{11}$	(2R,3R,4S,5S,6R)-2-{[(2S,3S,4S,5R)-3,4-Dihydroxy-2,5-bis(hydroxymethyl) oxolan-2-yl]oxy}-6-(hydroxymethyl) oxane-3,4,5-triol	100	65	4.0	185	−4.5	Sugarcane, sugar beet (Extraction process)	Na	Sweetener[d] Humectant[d] Flavor enhancer Color enhancer: Caramelization[d] Preservative[d] (Confectionery, candies, jams, beverages, table-top, etc)
Maltitol	$C_{12}H_{24}O_{11}$	4-O- α-d-Glucopyranosyl-d-glucitol	87	35	3.0	175	−5.5	High Maltose, Corn Syrup	Lower cooling effect	Sweetener Emulsifier Humectant Stabilizer Thickener Bulking agent
Lactitol	$C_{12}H_{24}O_{11}$	4-O-ß-1Galactopyranosyl-1glucitol	35	3	2.0	140	−13.9	Lactose	Slightly cooling effect	Sweetener Emulsifier Thickener
Isomalt	$C_{12}H_{24}O_{11}$	(2R,3R,4R,5R)-6-[[(2S,3R,4S,5S,6R)-3,4,5-Trihydroxy-6-(hydroxymethyl)-2-tetrahydropyranyl]oxy] hexane-1,2,3,4,5-pentol	54	2	0.45–0.65	39	−9.4	Sucrose	No cooling effects. Off-taste: bitter metallic	Sweetener Stabilizer Thickener Bulking agent Anti-caking agent Glazing agent

(continued)

Table 5.2 (continued)

Sweetener	Chemical formula[a]	Systematic (IUPAC) name[a]	% Relative sweetness vs. sucrose[a]	Glycemic index[b]	Caloric value (kcal/g)[c]	Solubility at 25 °C (g/100 ml)[c]	Heat of solution (cal/g)[c]	Source[d]	Taste[e]	Applications[e]
Sorbitol	$C_6H_{14}O_6$	D-Glucitol	58	4	2.6	235	−26.5	Glucose (Zymomonas mobilis)	Cooling effect	Sweetener Humectant Stabilizer Thickener Bulking agent Sequestrant
Mannitol	$C_6H_{14}O_6$	D-Mannitol	50	2	1.6	22	−28.9	Fructose (Fermentation: Leuconostoc *spp,* Lactobacilus spp	Cooling effect	Sweetener Humectant Stabilizer Thickener Bulking agent Anti-caking agent
Xylitol	$C_5H_{12}O_5$	D-erythro-pentitol	97	12	2.4	200	36.6	D-xylose (Yeast, Candida spp. Debaromyces spp.	Intense cooling sensation	Sweetener Emulsifier Humectant Stabilizer Thickener
Erythritol	$C_4H_{10}O_4$	1,2,3,4 butanetetrol, meso-erythritol	63	0	0.2	61	−43.9	Glucose (Fermentation, Moniella spp. Penicillium spp Pseudozyma tsukubaensis)	Cooling sensation	Sweetener Humectant Flavor enhancer

IUPAC International Union of Pure and Applied Chemistry, *Na* not applicable
[a]Chéron et al. [1]
[b]Livesey [5]
[c]Hartel et al. [4]
[d]Canada G [39]
[e]Grembecka [11]

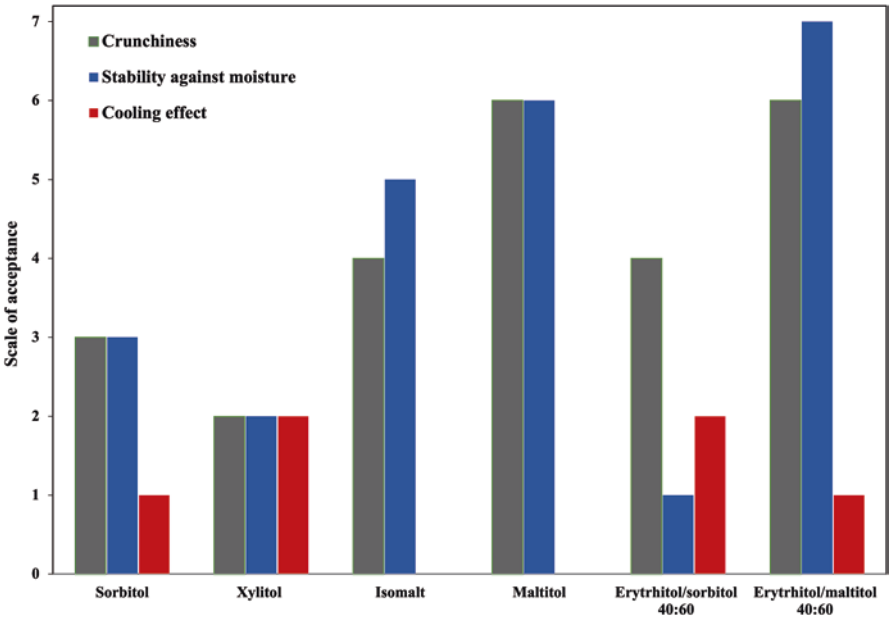

Fig. 5.3 Chewing gum coating parameters (crunchiness, stability against moisture, cooling effect) compared to sorbitol, xylitol, isomalt, maltitol, and mixtures of erythritol/sorbitol 40:60 and erythritol/maltitol 40:60. Scale level (high = 7, low = 0). (Adapted from Perko and Decock [23])

attracted attention because beyond providing sweetness, it is an additive with many vantages and potential applications in food as a stabilizer, moisturizer, texturizer, and flavor enhancer. It is suitable for dental products It is suitable for dental products due to D-tagatose does not cause tooth decay, which happens with sugar, once this rare sugar is not converted to acids by bacteria in the mouth, this acid slowly dissolves the enamel creating holes and cavities in the teeth. D-tagatose is a health promoter since D-tagatose is partially absorbed, only 15–20% is metabolized on small intestine [45], therefore has a minimal effect on blood glucose and insulin levels, being fermented on large intestine where are produced short-chain fatty acids (SCFA) which improve the gut health [14, 46].

D-tagatose was discovered in 1987 by Lobry de Bruyn and Van Ekenstein who were experimentally studying the transformations of reducing sugars in aqueous alkaline solutions [47]. Although just until 2001 D-tagatose was considered as a GRAS (Generally Recognized as Safe) additive by the U.S FDA (Food and Drug Administration), and the FAO (Food and Agriculture Organization) has suggested its use in food products after several clinical studies regarding D-tagatose effects and tolerance in humans [48–55]. As well the European Union, South Africa, New Zealand, and Australia also approved its consumption as a new ingredient [56]. D-tagatose can be found naturally in limited amounts in pineapples, apples, oranges [57], in gum exudate of the cacao tree (*Sterculia setigera*) [58], as a component of an oligosaccharide in lichens of the *Rocella* species [59] and also in dairy products

when milk is heated as UHT, pasteurized milk [60–62]. Industrially D-tagatose could be synthesized from lactose, a disaccharide formed by D-glucose and D-galactose present in milk or whey with both by the chemical and biological (enzymatic) processes. Firstly, the lactose is hydrolyzed, and D-glucose is eliminated, leaving only the D-galactose to be isomerized.

Under the chemical process, D-galactose is isomerized reacting with metal hydroxide (alkaline conditions) and neutralized with acid, after being filtered and purified, although this method involves the use of complex purification steps leading to the formation of unsafe chemical residues and a reduction in their sweetening properties representing high costs and disadvantages to producers. Then, several biological processes have been studied for years [63–65], since 1984, Izumori et al. [66] carried out the first enzymatic synthesis through the oxidation of D-galactitol using the enzyme sorbitol dehydrogenase from different microorganisms such as *Arthrobacter globiformis* ST48. Nowadays, the biological process more used is the isomerization of D-galactose using enzymes such as β-galactosidase and L-arabinose isomerase (L-AI) as biocatalysts, respectively, which is considered the greatest potential in use for the production of tagatose [67].

This sweetener is a 6-carbon monosaccharide with a chemical formula of $C_6H_{12}O_6$, it is known as an epimer of D-fructose due to both chemical structures are similar, only D-tagatose differs from D-fructose at the 4-carbon atom (Fig. 5.4). It is highly similar to sweet sucrose with 92% of its content in an aqueous solution of 10%, with a lower caloric value of 1.5 kcal/g, without after taste as other sweeteners, and prebiotic, antidiabetic, and obesity control properties. It is stable in a pH range between 2 and 7, very soluble in water (58% w/w at 21 °C) [14]. In Table 5.3 are shown the chemical and general properties of D-tagatose.

D-tagatose has a wide variety of uses in foods. As a low-calorie bulk sweetener make it a suitable ingredient for beverages (soft drinks) and dietary supplements. The flavor-enhancing properties of D-tagatose makes it a perfect and probable agent to mask the unpleasant taste of medicines or health products. Acu et al. [69]

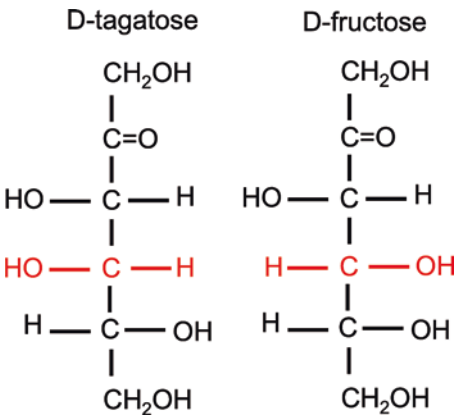

Fig. 5.4 Chemical structure of D-tagatose and the D-fructose epimer with fourth carbon as mirror image of D-tagatose in red color

Table 5.3 Chemical and general properties of D-tagatose [56, 68]

Properties	Value
Systematic (IUPAC) name	(3S,4S,5R)-1,3,4,5,6-Pentahydroxy-hexan-2-one
Chemical formula	$C_6H_{12}O_6$
Molecular weight	180.16 g/mol
Melting temperature	134 °C
pH	2–7
Solubility	160 g/100 mL at 20 °C
Relative sweetness	92% of sucrose
Odor	None
Color	White
Form	Crystalline solid
Caloric value	1.5 kcal/g
After taste	None
Maillard reaction and caramelization	Yes

IUPAC International Union of Pure and Applied Chemistry

evaluated the probiotic viability of ice cream made with frozen raspberry fruits, commercial raspberry and blackberry fruit purees, and tagatose as prebiotics. The authors observed that the ice cream samples maintained their probiotic properties during 120 days of storage and were generally well appreciated in terms of sensory properties by panelists.

In another research, Taylor et al. [70] used both partial and 100% sucrose replacer in cookies with tagatose. Rheological properties such as spread, hardness, and overall texture were similar when compared with control sweetened cookies (sucrose cookies), however, the authors compared the cookies having tagatose with cookies made with fructose which showed a softener dough cookie. Those results depend on sweetener solubility. D-tagatose solubility is slightly lower than sucrose at 20 °C (61% and 65%, respectively) while fructose is 88% at the same temperature. According to Manley [71], there are two types of cookies, hard and soft cookies, the difference is the existence or not of long chains of gluten that give the dough extensibility. The gluten development in the dough is directly related to water availability in the process. More water available allows wheat flour proteins hydration, consequently enabling the formation of the gluten chains. The dough will exhibit viscoelastic properties giving rise to hard cookies without spreading the dough too far; therefore, cookies preserve their round shape on the baking sheet; however, when sugar amount is high and consequently highly soluble in water, water availability decreases, and gluten is not able to be developing resulting in softer cookies, due to the dough does not achieve elasticity, and it spreads during baking creating irregular sizes. Fat excess also interferes with gluten development, coating proteins in flour responsible for forming the gluten, making an impermeable layer on the dough. Low-fat content results in strong doughs. Thus gluten, water, sugar, and fat play an essential role in the dough [71].

Tagatose also improved the color of the cookies, based on high scores data from panelists who liked the brown color of cookies with 100% tagatose cookies better than the control [70], this is due to tagatose participation in Maillard reaction and caramelization. Although the sweetness of 100% of tagatose was perceived, the overall likeness was acceptable for panelists. D-Tagatose is also useful as a texturizer and stabilizer enhancing storage stability by anti-blooming effects on chocolate when compared to sucrose and maltitol added to chocolate. Also, the acceptance of chocolates produced with tagatose was superior to stevia added chocolate, according to consumers responses (n = 219). Tagatose was scored positively as more similar to sucrose added chocolate rather than stevia, also overall liking was better on texture, bitterness, duration of aftertaste and intensity of aftertaste [72]. In summary, Table 5.4 showed some applications of tagatose in different food and non-food products.

5.2.4 Steviol Glycosides

Among all-natural sweeteners, steviol glycosides SGs (stevioside and rebaudioside-A) are popularly known as zero-caloric intense sweetening compounds of natural origin [80]. Stevia rebaudiana Bertoni (Stevia) is a perennial herb of the Arteraceae family, native to South America. Stevia leaves contain steviol glycosides that have been used as a sweetener in South America for centuries and today their consumption has spread throughout the world [81].

Table 5.4 Applications of tagatose in food and non-food products

Properties	Industrial use	Product	References
Sweetener	Food	Frostings	[56]
Probiotic and sweetener	Food	Ice cream	[69]
Probiotic and sweetener	Food	Yogurt	[73]
Sweetener and flavor enhancer	Food	Beverages	[74, 75]
Sweetener and flavor enhancer	Food	Hard candies, soft candies and jelly	[56, 76]
Texturizer, stabilizer, and sweetener	Food	Chocolates	[72, 77, 78]
Sweetener, color enhancer, texturizer	Food	Baked food, cookies, cake	[70]
Sweetener and texturizer	Food	Chewing gum	[56]
Sweetener	Food	Breakfast cereals	[79]
Sweetener and flavor enhancer	Cosmetic, personal hygiene	Toothpaste, mouthwash, cosmetics	[67]
Sweetener	Drug	Oral antibiotics, Chewable flavored antibiotic tablets	[14]
Flavor enhancer	Cosmetic	Flavored lipstick	[46]

According to Singh et al. [82], stevia rebaudiana (Bertoni) is a plant widely known in the Amambay region of Paraguay and used as a natural sweetener and in traditional local medicine. In addition to the sweetening power of stevia, it has bioactive compounds with anti-inflammatory, immunomodulatory, antimicrobial, cardiovascular, anticancer and antidiabetic properties [80]. Stevia extracts have been widely used to elevate sweetness levels in foods and the sweet taste of stevia is mainly attributed to various glycosides such as stevioside, rebaudioside-A, -B, -C, -D, -E and dulcoside-A. Among them, stevioside, and rebaudioside-A can be extracted with hot water [80, 83, 84].

In the last two decades, stevia rebaudiana bertoni leaves have attracted much interest not only as a non-caloric sweetener known as steviol glycosides, but also as a valuable by-product [85]. In fact, the use of stevia rebaudiana bertoni leaf extracts as a low-calorie sweetener is trending upward for beverages, and these extracts are sources of bioactive compounds (e.g., polyphenols, chlorophylls, carotenoids, and ascorbic acid) with antimicrobial properties and antioxidants [86]. In this correlation of interest and properties, stevia rebaudiana bertoni leaves have attracted much interest from researchers and the food industry not only as a non-caloric sweetener, but also as a valuable by-product [85].

Stevia rebaudiana is a perennial shrub, being a member of the 950 genera of the Asteraceae family. To date, more than 150 species of stevia are known, with stevia rebaudiana (Bertoni) being the one that differs from other species due to its high degree of sweetness [87]. Stevia rebaudiana is a short-day plant that grows up to 1 m tall. Its leaves have an elliptical shape and a length between 2 and 3 cm with an alternating arrangement. The stem of the plant is fragile and allows a condition to be broken easily. In addition, its root system is extensive. The flowers have a white coloration and a pale purple color in the throat of the same. The flowers are smaller than the leaves and are arranged in the form of small corymbs [82].

Stevia can be found naturally in subtropical regions of semi-humid conditions at a height between 200 and 400 m above sea level. For its natural growth, rainfall of around 1500–1800 mm and a wide temperature range between −6 and 43 °C are required [8]. According to Yadav et al. [8], the first stevia crops were domesticated in 1968 in Japan, allowing in the 1970s stevioside, from the Stevia leaf, to become a commercially important sweetener and food supplement. Currently, Stevia has been adopted and commercialized by several countries such as Brazil, Korea, United Kingdom, China, and Malaysia.

From the leaves of the evergreen stevia rebaudiana Bertoni shrub, indigenous people obtained extracts that were used as a sweetener for various foods and beverages and in medicines [88]. Steviol glycoside extracts of high purity (≥95%) after several studies have been approved for use as a food sweetener in several countries and regions, including the European Union and the United States. the sweetening property is a result of the presence of natural plant constituents known as steviol glycosides (SGs) [89, 90].

The SGs obtained from the plant are four-ring diterpenes composed of an aglycone backbone called steviol to which various numbers and types of sugars are attached (Fig. 5.5a). Currently, >40 SGs have been identified, stevioside (CAS No.

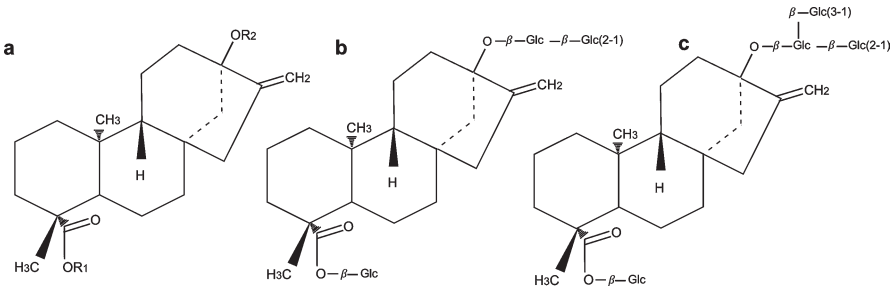

Fig. 5.5 Backbone structure of SGs (**a**), Stevioside (**b**), and Rebaudioside A (**c**). (Adapted from Anker et al. [81])

57817-89-7, 4–13% wt:wt, Fig. 5.5b) and rebaudioside A (CAS No. 58543-16, Reb A—2–4% wt:wt, Fig. 5.5c) being the most abundant glycosides in stevia rebaudiana leaves. Stevioside and Reb A are non-caloric compounds with a sweetening capacity of around ~200–300 times more than 0.4 M sucrose and are chemically very similar, differing only by an additional glucose fraction in Reb A. In general, SGs differ only in the number and type of monosaccharides attached to the aglycone [81].

According to Puri et al. [91], steviol is a chemically and thermally stable compound, which does not lose its sweetness index of 300, thus allowing its wide use in various industries with possible application with greater use in the food industry. Consequently, Brahmachari et al. [88] cited that steviol has a wide acceptance of use worldwide, considered the "third" glycogen in the world; in addition, they report that there is no evidence of side effects related to its use in humans. On the other hand, Azarpazhood et al. [92] informed that stevioside and rebaudioside A have economic advantages when compared to other glycosides derived from this plant.

In addition to the known facts of its sweetening power, there is scientific evidence that stevia has medicinal benefits, including nematicide, antioxidant, wound healing activity, antiviral, anti-inflammatory, antidiabetic, and kidney protection [93–96]. Stevioside is non-toxic and its therapeutic value consists of the possibility of replacing sugar and the ability of this compound to stimulate insulin secretion in the pancreas in the treatment of diabetes and other disorders of carbohydrate metabolism [97].

Interestingly, stevia-derived glycosides are non-carcinogenic, non-mutagenic, non-teratogenic, and do not induce acute or subacute toxicity [98]. It is important to highlight that those studies show an improvement in cholesterol regulation with the frequent use of stevia, in addition to presenting antiviral properties and producing a positive therapeutic effect in neuralgia treatments [99]. Researchers have associated the benefits of Stevia mainly to its nutritional composition, since it has a good source of carbohydrates, protein, and crude fiber, thus promoting well-being and consequently reducing the risk of certain diseases. In Table 5.5 it is possible to find the nutritional information of stevia reported by several authors.

Table 5.5 Approximate analysis of dried stevia leaves

Components	Comment on					
	Jyoti et al. [100]	Goyal et al. [101]	Serio [102]	Abou-Arab et al. [103]	Lemus-Mondaca et al. [104]	Kaushik et al. [105]
Moisture	7	4.65	Nd	5.37	Nd	7.7
Carbohydrates	52	Nd	53	61.9	35.2	Nd
Protein	10	11.2	11.2	11.40	20.4	12
Fat	3	1.9	5.6	3.73	4.34	2.7
Crude Fiber	18	15.2	15	15.5	Nd	Nd
Ash	11	6.3	Nd	7.41	13.1	8.4

Nd not determined

Consequently, it is possible to observe that there are no significant changes in the composition reported by the researchers, thus allowing the possible application of the extracts as dietary supplements.

In relation to the numerous results found in the literature that report the benefits of stevia, it has a potential for use as a source of natural antioxidants in the cosmetic and food industries. It should be noted that even though stevia did not present levels of toxicity and was accepted as a GRAS food, several authors still recommended caution regarding the use of extracts before further toxicological studies are carried out due to the cytotoxicity of ethanolic and aqueous glycol extracts [106]. Table 5.6 shows the wide use of stevia at an industrial level, focusing on the use of elements of natural origin to reduce the impacts that synthetics can bring. It is clear that this natural sweetener has been widely used in the food and beverage industry as well as the pharmaceutical industry. Its use and acceptance at the medicinal level is due to studies that have shown promising benefits against diabetes, obesity, hypertension, cancer, tooth decay, oxidative and antimicrobial stress.

5.2.5 Glycyrrhizin

The glycyrrhizin (18 β-glycyrrhetinic-acid-3-O-[β-d-glucuronopyranosyl-(1 → 2)-β-d-glucuronopyranoside], GL), which is more correctly called glycyrrhizinic acid [117], is a kind of natural edulcorant as well as one component in Oriental medicine. The hydrophobic backbone is built by a triterpene called glycyrrhetinic acid [118]. GL is the main component of licorice extract (*Glycyrrhiza glabra*), being commonly used as a sweetener. GL is until 150 times sweeter than sucrose [119]. Moreover, it exhibits low toxicity and is therefore used as a sweetener. However, the recommended daily consumption is less than 0.229 mg glycyrrhizin/kg body weight/day [120].

According to Zhang et al. [120], licorice extract is extensively used worldwide as a natural sweetener, pharmaceutical agent, and dietary supplement. Besides that, glycyrrhizin is generally regarded as safe (GRAS) in the USA, European, and other

Table 5.6 Principal industrial applications of stevia

Properties	Industrial use	Product	References
Antioxidant	Food	Wheat bread	[107]
Improve concentration of phenolic compounds and antioxidant	Food	Juice	[108]
Antioxidant	Food	Yogurts	[109]
Organoleptic properties	Food	Oatmeal raisin cookies	[110]
Sensory acceptability	Food	Dairy dessert	[111]
Biosurfactant	Food	Small micelles with size of around 4.70 nm	[112]
Anti-tumor property	Medical	Na	[113]
Anticancer activity (gastrointestinal cancer cells)	Medical	Na	[114]
Colon cancer cell lines	Medical	Na	[115]
Reducing glycemic index	Medical/ Food	Bakery (Muffins)	[116]

Na not applicable

countries [121]. On the other hand, in the literature it is possible to find evidence indicating that glycyrrhizin has other differentiated biological activities when compared to other sweeteners, thus, it is possible to cite its anti-inflammatory [122], antioxidant [123], antiviral [124], antitumor [125] and hepatoprotective [126] activity.

Native to Asia and the Mediterranean region, licorice (*Glycyrrhiza glabra*) is a tall shrub in the family Leguminosae, thus the genus Glycyrrhiza Linn. (Fabaceae) is composed of approximately 20 species [127]. Although most commercial licorice is extracted from varieties of G. glabra grown in southern and central Europe (var. *typica*), central and southern Russia (var. *glandulifera*) and Iran and Iraq (var. *violacea*). Licorice also grows in the United States (var. *lepidota*) and England (var. *typica*), but neither represents a significant contribution to world production [128]. According to Isbrucker et al. [128] the fresh root contains about 20% of water-soluble extractives, and around 3–5% of the root is composed of glycyrrhizin, present as a mixture of potassium and calcium salts. Licorice root extract contains between 10% and 25% glycyrrhizin as the primary active ingredient. Minor constituents which may also confer some pharmacological activities include liquiritigenin, isoliquiritigenin, and their corresponding aglycones [129].

Chemically, glycyrrhizin is composed of hydrophobic aglycone 18β-glycyrrhetinic acid ($C_{30}H_{46}O_4$, 470.68 g/mol) bound at position C-3 via an ether bond to a sugar chain composed of two glucuronic acid units, making the molecule amphiphilic (Fig. 5.6) [118]. The acidic group at the C-20 position of glycyrrhetinic acid significantly influences the amphiphilicity of the whole molecule depending on the pH value. The polyvalent weak acid group also determines the solubilizing properties of the molecule. According to Matsuoka et al. [118], glycyrrhizin is insoluble at low and native pH ($pH \leq 4.5$). In contrast, at pH 4.5–5.0,

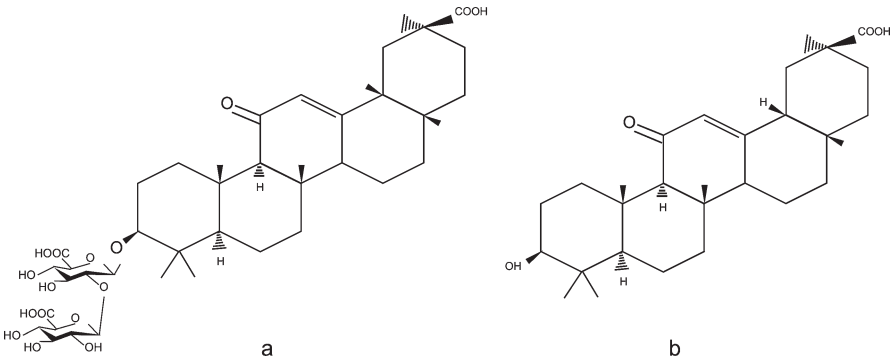

Fig. 5.6 Chemical structures of Glycyrrhizinic (**a**) and Glycyrrhizinic acid (**b**). (Adapted from Graebin [117])

the anisotropic structure of glycyrrhizin led to the formation of rod-like micelles and fibrils with a height of 2.5 nm and periodicity of 9 nm that self-assembled either at the interface or within the continuous phase into a fibrillary network at concentrations of 5.3–10 mmol/L [130]. Upon increasing concentrations, this is most probably due to the deprotonated acidic bound groups to the backbone at opposite sites, which causes the loss of the clear amphiphilic structure and moreover induces repulsion effects between different glycyrrhizin molecules [131, 132].

According to Hosseini et al. [133], the sweetening power of glycyrrhizin allows it to be commonly used in the agro-food industries and due to its versatility, saponite has its scope in traditional Chinese, Tibetan and Indian medicinal preparations, while glycyrrhetinic acid is used in the treatment of chronic liver diseases, being marketed in Japan, China, Korea, Taiwan, Indonesia, India and Mongolia [117].

The extracts collected or the powder prepared predominantly from the roots and rhizomes usually hold pharmacological importance. Thus, Wang and Nixon [134] evaluated the potential anticancer effects of licorice extract and glycyrrhizable compounds by establishing that licorice polyphenols induce apoptosis in cancer cells. Thus, these and other activities of licorice infer a suggested justification for combinations of agents in preventive clinical trials. On the other hand, Ruschitzka et al. [135] evaluated the effects of glycyrrhizin (50 mg/kg, i.p., twice a day for 7 days) on nitric oxide production and vascular endothelin response were monitored in male Wistar rats. The aortic endothelial nitric oxide response was significantly inhibited by glycyrrhizin treatment, as indicated by reduced aortic tissue nitrate concentrations and decreased endothelial nitric oxide synthase protein levels.

Recently, Gomaa and Abdel-Wadood [129] reported scientific evidence on the use of glycyrrhizin and licorice extract as a fighting agent against COVID-19. Thus, the researchers conclude that in relation to the literature, licorice extract has capacity against COVID-19, but that randomized clinical trials are needed to reach an accurate conclusion [136]. The sweet-tasting yellow licorice root extract, which is considered a blend with various bioactive constituents (flavonoids and various phenolic acids), has a variety of benefits and applications [137, 138]. For example,

polyphenolic compounds like tannins and flavonoids, which are found in abundance in licorice extract, are radical scavengers. In general, naturally occurring phytochemical substances with antioxidant/free radical scavenging characteristics based on their existing structure in plant extracts are reported to have corrosion inhibitory capacity [139]. Licorice being a traditional Chinese medicinal herb began to be used in industrial applications with wide impact in the food and cosmetics industries [140].

Licorice and licorice derivatives are generally recognized as safe (GRAS) for use in food by the US FDA (21 CFR 184.1408 [141]). In Table 5.7, the maximum authorized concentrations for the use of licorice (glycyrrhizin) and its derivatives for use in food are established.

Regarding the limitations established by the FDA, it is possible to find in the literature several applications of GL in the food industry, as can be seen in Table 5.8, which presents the wide use of the sweetener at an industrial level.

However, as licorice application scenarios continue to be discovered, the worldwide demand for licorice is also increasing and the problem of supply of licorice resources has arisen [136]. China is a major producer of licorice and its products and is extensively involved in the international licorice trade. However, at the same time, China faces depletion of licorice resources and related international trade competitive problems. Thus, Han et al. [152] conclude that still, the main focus is on the use of licorice extracts, while there is little attention given to licorice residues. Further inferring that it is necessary to pay attention to the transformation of licorice waste into by-products and increase the added value to promote a circular economy in addition to promoting cooperation and exchange between the main producers to improve the industrial chain and achieve the sustainable use of resources.

GL has wide acceptance and industrial use around the world. Therefore, given the properties of licorice and its active constituents, it is suggested that their potential roles be evaluated by their effects on both food and medicine. However, further studies are needed to confirm these effects.

Table 5.7 Maximum authorized concentrations of glycyrrhizin in foods regulated by Food and Drug Administrations (FDA) [141]

Food category	Maximum allowable levels of glycyrrhizin	Functional use
Baked goods	0.05	FE, FA
Alcoholic beverages	0.1	FE, FA, SA
Non – alcoholic beverages	0,15	FE, FA, SA
Chewing gum	1.1	FE, FA
Hard candy	16.0	FE, FA
Soft candy	3.1	FE, FA
Herbs and seasonings	0.15	FE, FA
Plant protein products	0.15	FE, FA
Vitamins or mineral dietary supplements	0.5	FE, FA

FE flavor enhancer, *FA* flavoring agent, *SA* surface-active agent

Table 5.8 Main activities of glycyrrhizin and the action mechanisms

Properties	Industrial use	Product	References
Antioxidant capacity	Food	Preserved the quality of Japanese sea bass fillets	[142]
Antioxidant activity	Food	Precooked pork patties	[143]
Antimicrobial activity	Food	Meat products (fresh pork and ham products)	[144]
Antibacterial effects	Food	Milk and labneh	[145]
Sensorial and rheological properties	Food	Probiotic product (ROSALACT®)	[146]
Bioavailability	Food	Nano-emulsions containing Glycyrrhizin	[147]
Low-sugar flavored drinks	Food	Milk products	[148]
Anti-inflammatory and anti-ulcer	Medical	Na	[149]
Preventing and treating dental caries, periodontitis, gingivitis, candidiasis, recurrent aphthous ulcer, and oral cancer.	Medical	Na	[150]
Probiotic and prebiotic activities	Medical	Na	[151]

Na Not applicable

5.2.6 Thaumatin

Thaumatin is it a mixture of sweet proteins (thaumatin I and II) extracted from the arils of the fruit of *Thaumatococcus daniellii* (Benth) a West African rainforest shrub. It has a high intense sweetener about 2000 times sweeter than sugar, a potent flavor/aroma enhancer, and has the ability to mask unwanted aftertaste from numerous substances, including artificial sweeteners. It was first documented in 1855 by scientist W.F. Daniell, who described it as a powerful sweetener and flavor and aroma enhancer in local foods and beverages [9, 153, 154].

While there are others sweet proteins which have been identified and isolated from tropical plants such as brazzein [155], pentadin [156], curculin [157], and monellin [158], thaumatin has been most studied [159], however, a large-scale production, cost, quality, and acknowledged applications are currently some limitations to commercialize this sweet protein [160, 161].

Thaumatin is composed of a sequence of 207 amino acids, and it is digested by the human body and animals following the normal metabolism of other natural proteins, for that reason thaumatin is considered GRAS by the U.S FDA and by the European Union under the code E957. Its properties include, odorless, flavor enhancer, flavor masker (bitter or unpleasant taste), stable at 120 °C and both acid and alkaline pH environments (from 2.0 to 10 at room temperature), water soluble, slow onset sweetness but sweet aftertaste, and 4 kcal. g^{-1} [159].

In spite its high-intensity sweetness and flavor enhancer and masking effect, not several data were found in the literature on the use of thaumatin in food applications. Until now the applications found of thaumatin added were to Skyr yogurt with

mango pulp by Pereira et al. [162] which observed the sweetener had better acceptance on the sensory profile above stevia from consumers panelists results, also stevia/thaumatin blend (ratio 1:1). Thaumatin did not affect the texture and syneresis of natural skyr yogurt when compared to sucrose formulation of yogurt. Firsov et al. [163] used thaumatin in salted, pickled tomatoes stored through 6 months, and processed tomatoes. Thaumatin showed high stability during salting, acidic (at a pH = 3.1) and storage. Its amount was similar to the content added to fresh tomatoes before processing. Therefore, both salt and acid environments do not influence on thaumatin sweetener properties. Although both salted and pickled tomatoes had a common thaumatin after taste, the overall likeness of pickled tomatoes was scored by panelists as better compared to fresh and salted tomatoes after 6 months of storage, which means thaumatin was a flavor enhancer in acid conditions.

A reducing sugar concentration from 17% of sucrose in strawberry petit Suisse cheese was achieved using a combination of thaumatin/sucralose (at ratio 2:1) at 0.018% with a potency 157.40 reaching the same sweetness sensation as sucrose evaluated by Sousa et al. [164] reducing calories. Authors evaluated four formulations as sucralose, sucralose/acesulfame-K (4:1), thaumatin/sucralose (2:1) and cyclamate/saccharin (1:1). Even results showed cyclamate/saccharin (at ratio 1:1) were the sweeteners with the highest concentration 0.349%, thaumatin and sucralose (at ratio 2:1) might reach the same sweetness sensation.

5.3 Conclusions

Food applications of sweeteners require reformulation in manufacturing and food production to address crucial and technical challenges.

Despite sugar reduction or total replacement, its impact is not only on sweetness but also overall texture, color, taste, and flavor of products; however, several sweeteners can also act as a bulking and stabilizer agent and have culinary properties that can affect positively baked goods, candies, glazes, dairy products, and others.

Characteristics such as after taste are mainly associated with the natural plants extracted sweeteners such as steviol glycosides, glycyrrhizin, and thaumatin; however, the advantage to providing zero calories and zero glycemic indexes without impact on the human body is desirable for low caloric food applications. Sugar alcohols and rare sugars are low caloric with potential uses; however, their doses should be regulated. Water solubility is a concern that affects specially baked products to choose the sweetener, however blends or mixtures of them improve product characteristics with better sensorial acceptance. Nevertheless, sugar alcohols provide a cooling effect to products specially mixed with other flavors.

In general, the introduction of food additives such as sweeteners to the market has provided new opportunities to study the chemical interactions of different ingredients and their impact on human health. More studies need to be done to reach other food products.

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References

1. Chéron JB, Marchal A, Fiorucci S. Natural sweeteners. Encyclopedia of food chemistry. Amesterdam: Elsevier; 2018. p. 189–95.
2. Biancardi EJ, Mitchell M, Panella LW, Lewellen RT, Stevanato P. Chapter 6. Sugar beet. In: Bradshaw J, editor. Root and tuber crops. New York: Springer; 2015. p. 173–219.
3. Inglett GE. A history of sweeteners-natural and synthetic. J Toxicol Environ Health. 1976;2(1):189–206.
4. Hartel RW, Von Elbe JH, Hofberger R. Confectionery science and technology. In: Hartel RW, Von Elbe JH, Hofberger R, editors. Confectionery science and technology. 1st ed. Cham: Springer Nature Switzerland; 2018. p. 1–517.
5. Livesey G. Health potential of polyols as sugar replacers, with emphasis on low glycaemic properties. Nutr Res Rev. 2003;16(2):163–91.
6. O'Brien NL. In: O'Brien Nabors L, editor. Alternative sweeteners. 4th ed. Boca Raton: CRC Press; 2012. p. 1–587.
7. García-Almeida JM, Fdez GMC, Alemán JG. Una visión global y actual de los edulcorantes. Aspectos de regulación. Nutr Hosp. 2013;28(4):17–31.
8. Yadav SK, Guleria P. Steviol glycosides from stevia: biosynthesis pathway review and their application in foods and medicine. Crit Rev Food Sci Nutr. 2012;52(11):988–98.
9. Lindley MG. Natural high-potency sweeteners. In: O'Donnell K, Kearsley MW, editors. Sweeteners and sugar alternatives in food technology. 2nd ed. Oxford: John Wiley & Sons, Ltd.; 2012. p. 185–212.
10. Chattopadhyay S, Raychaudhuri U, Chakraborty R. Artificial sweeteners – a review. J Food Sci Technol. 2014;51(4):611–21.
11. Grembecka M. Sugar alcohols—their role in the modern world of sweeteners: a review. Eur Food Res Technol. 2015;241(1):1–14.
12. Kagawa, University, Miki J. International Society of Rare Sugars. 2019. Available from: https://www.isrs.kagawa-u.ac.jp/society.html.
13. Mijailovic N, Nesler A, Perazzolli M, Aït Barka E, Aziz A. Rare sugars: recent advances and their potential role in sustainable crop protection. Molecules. 2021;26(6):1720.
14. Levin GV. Tagatose, the new GRAS sweetener and health product. J Med Food. 2002;5(1):23–36.
15. Scott SK, Rabito FA, Price PD, Butler NN, Schwartzbaum JA, Jackson BM, et al. Comorbidity among the morbidly obese: a comparative study of 2002 U.S. hospital patient discharges. Surg Obes Relat Dis. 2006;2(2):105–11.
16. Yoshida H, Hayashi J, Sugahara T. Studies on free sugars, free sugar alcohols and organic acids of wild mushrooms. J Jpn Soc Food Sci Technol. 1987;33(6):426–33.
17. Rzechonek DA, Dobrowolski A, Rymowicz W, Mirończuk AM. Recent advances in biological production of erythritol. Crit Rev Biotechnol. 2018;38(4):620–33.
18. Bernt WO, Borzelleca JF, Flamm G, Munro IC. Erythritol: a review of biological and toxicological studies. Regul Toxicol Pharmacol. 1996;24(2 II):191–7.
19. Moon HJ, Jeya M, Kim IW, Lee JK. Biotechnological production of erythritol and its applications. Appl Microbiol Biotechnol. 2010;86(4):1017–25.

20. Kumar S, Tyagi PK, Gola D, Mishra AK, Arya A. Plant-based sweeteners and their applications in modern lifestyle. In: Husen A, Bachheti KR, Archana B, editors. Non-timber forest products. Cham: Springer Nature Switzerland; 2021. p. 75–103.
21. Regnat K, Mach RL, Mach-Aigner AR. Erythritol as sweetener—wherefrom and whereto? Appl Microbiol Biotechnol. 2018;102(2):587–95.
22. Röper HV, Goossens JB. Erythritol, a new raw material for food and non-food applications. Starch – Stärke. 1993;45(11):400–5.
23. Perko R, Decock P. Part three reduced-calorie bulk sweeteners erythritol. In: Helen M, editor. Sweeteners and sugar alternatives in food technology. Iowa: Blackwell Publishing Ltd.; 2006. p. 151–76.
24. Boesten DMPHJ, den Hartog GJM, de Cock P, Bosscher D, Bonnema A, Bast A. Health effects of erythritol. Forum Nutr. 2015;14(1):3–9.
25. Carocho M, Morales P, Ferreira ICFR. Natural food additives: quo vadis? Trends Food Sci Technol. 2015;45(2):284–95.
26. Storey D, Lee A, Bornet F, Brouns F. Gastrointestinal tolerance of erythritol and xylitol ingested in a liquid. Eur J Clin Nutr. 2007;61(3):349–54.
27. Oku T, Okazaki M. Laxative threshold of sugar alcohol erythritol in human subjects. Nutr Res. 1996;16(4):577–89.
28. Tetzloff W, Dauchy F, Medimagh S, Carr D, Bär A. Tolerance to subchronic, high-dose ingestion of erythritol in human volunteers. Regul Toxicol Pharmacol. 1996;24(2 II):286–95.
29. Carocho M, Morales P, Ferreira ICFR. Sweeteners as food additives in the XXI century: a review of what is known, and what is to come. Food Chem Toxicol. 2017;107:302–17.
30. De Cock P, Bechert CL. Erythritol. Functionality in noncaloric functional beverages. Pure Appl Chem. 2002;74(7):1281–9.
31. O'Donnell K, Kearsley M. Sweeteners and sugar alternatives. In: O'Donnell K, Kearsley M, editors. Sweeteners and sugar alternatives in food technology. 2nd ed. Oxford: Wiley-Blackwell; 2012. p. 1–471.
32. Fry J. Natural low-calorie sweeteners. In: Baines D, Seal R, editors. Natural food additives, ingredients and flavourings. 1st ed. Cambridge: Woodhead Publishing; 2012. p. 41–5.
33. Lin SD, Lee CC, Mau JL, Lin LY, Chiou SY. Effect of erythritol on quality characteristics of reduced-calorie danish cookies. J Food Qual. 2010;33(Suppl. 1):14–26.
34. Laguna L, Primo-Martín C, Salvador A, Sanz T. Inulin and erythritol as sucrose replacers in short-dough cookies: sensory, fracture, and acoustic properties. J Food Sci. 2013;78(5):S777–84.
35. Akesowan A. Quality of reduced-fat chiffon cakes prepared with erythritol-sucralose as replacement for sugar. Pak J Nutr. 2009;8(9):1383–6.
36. Martínez-Cervera S, Salvador A, Sanz T. Comparison of different polyols as total sucrose replacers in muffins: thermal, rheological, texture and acceptability properties. Food Hydrocoll. 2014;35:1–8.
37. Spies RD, Hoseney RC. Effect of sugars on starch gelatinization. Cereal Chem. 1982;59:128.
38. Rice T, Zannini E, Arendt EK, Coffey A. A review of polyols–biotechnological production, food applications, regulation, labeling and health effects. Crit Rev Food Sci Nutr. 2020;60(12):2034–51.
39. Canada G. Sugar alcohols (polyols) and polydextrose used as sweeteners in foods – food safety – health Canada. 2005. Available from: https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/sugar-substitutes/sugar-alcohols-polyols-polydextrose-used-sweeteners-foods-food-safety.html.
40. Grembecka M. Sugar alcohols. Encyclopedia of analytical science. Amsterdam: Elsevier Ltd.; 2019. p. 290–9.
41. Tuncer D, Onen A, Yazici AR. Effect of chewing gums with xylitol, sorbitol and xylitol-sorbitol on the remineralization and hardness of initial enamel lesions in situ. Dent Res J. 2014;11(5):537–43.

42. Oza S, Patel K, Bhosale S, Mitra R, Gupta R, Choudhary D. To determine the effect of chewing gum containing xylitol and sorbitol on mutans streptococci and Lactobacilli count in saliva, plaque, and gingival health and to compare the efficacy of chewing gums. J Int Soc Prev Community Dent. 2018;8(4):354–60.
43. Kawanabe J, Hirasawa M, Takeuchi T, Oda T, Ikeda T. Noncariogenicity of erythritol as a substrate. Caries Res. 1992;26(5):358–62.
44. Jeon Y, Oh J, Cho MS. Formulation optimization of sucrose-free hard candy fortified with cudrania tricuspidata extract. Foods. 2021;10(10):1–17.
45. Bertelsen H, Jensen BB, Buemann B. D-tagatose--a novel low-calorie bulk sweetener with prebiotic properties. World Rev Nutr Diet. 1999;85:98–109.
46. Kim P. Current studies on biological tagatose production using L-arabinose isomerase: a review and future perspective. Appl Microbiol Biotechnol. 2004;65(3):243–9.
47. Karabinos JV. Psicose, sorbose and tagatose. Adv Carbohydr Chem. 1952;7(C):99–136.
48. Lee A, Storey DM. Comparative gastrointestinal tolerance of sucrose, lactitol, or D-tagatose in chocolate. Regul Toxicol Pharmacol. 1999;29(2 I):78–82.
49. Buemann B, Toubro S, Raben A, Blundell J, Astrup A. The acute effect of D-tagatose on food intake in human subjects. Br J Nutr. 2000;84(2):227–31.
50. Buemann B, Toubro S, Astrup A. D-tagatose, a stereoisomer of D-fructose, increases hydrogen production in humans without affecting 24-hour energy expenditure or respiratory exchange ratio. J Nutr. 1998;128(9):1481–6.
51. Buemann B, Toubro S, Raben A, Astrup A. Human tolerance to a single, high dose of D-tagatose. Regul Toxicol Pharmacol. 1999;29(2 I):66–70.
52. Buemann B, Toubro S, Astrup A. Human gastrointestinal tolerance to D-tagatose. Regul Toxicol Pharmacol. 1999;29(2 I):71–7.
53. Boesch C, Ith M, Jung B, Bruegger K, Erban S, Diamantis I, et al. Effect of oral D-tagatose on liver volume and hepatic glycogen accumulation in healthy male volunteers. Regul Toxicol Pharmacol. 2001;33(2):257–67.
54. Saunders JP, Donner TW, Sadler JH, Levin GV, Makris NG. Effects of acute and repeated oral doses of D-tagatose on plasma uric acid in normal and diabetic humans. Regul Toxicol Pharmacol. 1999;29(2 I):57–65.
55. Normén L, Lœrke HN, Jensen BB, Langkilde AM, Andersson H. Small-bowel absorption of D-tagatose and related effects on carbohydrate digestibility: an ileostomy study. Am J Clin Nutr. 2001;73(1):105–10.
56. Roy S, Chikkerur J, Roy SC, Dhali A, Kolte AP, Sridhar M, et al. Tagatose as a potential nutraceutical: production, properties, biological roles, and applications. J Food Sci. 2018;83(11):2699–709.
57. Surapureddi SRK, Ravindhranath K, Kumar GSS, Chiliveri P, Sappidi SR. High resolution and high throughput analytical methods for D-tagatose and process related impurities using capillary electrophoresis. Anal Biochem. 2020;609(113981):1–10.
58. Hirst EL, Hough L, Jones JKN. Composition of the gum of Sterculia setigera: occurrence of D-tagatose in nature. Nature. 1949;4135:163–77.
59. Lindberg B. Studies on the chemistry of lichens. VIII. Investigation of a dermatocarpon and some Roccella species. 1955. Report No.: 6.
60. Mendoza MR, Olano A, Villamiel M. Chemical indicators of heat treatment in fortified and special milks. J Agric Food Chem. 2005;53(8):2995–9.
61. Adachi S. Formation of lactulose and tagatose from lactose in strongly heated milk. Nature. 1958;181(4612):840–1.
62. Troyano E, Martinez-Castro I, Olano A. Kinetics of galactose and tagatose formation during heat-treatment of milk. Food Chem. 1992;45(1):41–3.
63. Xu Z, Li S, Fu F, Li G, Feng X, Xu H, et al. Production of D-tagatose, a functional sweetener, utilizing alginate immobilized Lactobacillus fermentum CGMCC2921 cells. Appl Biochem Biotechnol. 2012;166(4):961–73.

64. Izumori K, Tsuzaki K. Production of d-tagatose from d-galactitol by Mycobacterium smegmatis. J Ferment Technol. 1988;66(2):225–7.
65. Manzoni M, Rollini M, Bergomi S. Biotransformation of D-galactitol to tagatose by acetic acid bacteria. Process Biochem. 2001;36(10):971–7.
66. Izumori K, Miyoshi T, Tokuda S, Yamabe K. Productoin of D-tagatose from dulcitol by Arthrobacter globiformis. Appl Environ Microbiol. 1984;48(5):1055–7.
67. Ravikumar Y, Ponpandian LN, Zhang G, Yun J, Qi X. Harnessing L-arabinose isomerase for biological production of D-tagatose: recent advances and its applications. Trends Food Sci Technol. 2021;107:16–30.
68. Roh HJ, Kim P, Park YC, Choi JH. Bioconversion of D-galactose into D-tagatose by expression of L-arabinose isomerase. Biotechnol Appl Biochem. 2000;31(1):1.
69. Acu M, Kinik O, Yerlikaya O. Probiotic viability, viscosity, hardness properties and sensorial quality of synbiotic ice creams produced from goat's milk. Food Sci Technol (Brazil). 2021;41(1):167–73.
70. Taylor TP, Fasina O, Bell LN. Physical properties and consumer liking of cookies prepared by replacing sucrose with tagatose. J Food Sci. 2008;73(3):S145–51.
71. Manley D. Part III types of biscuits. In: Manley D, editor. Technology of biscuits, crackers and cookies. 3rd ed. Boca Raton: CRC Press; 2000.
72. Lagast S, De Steur H, Schouteten JJ, Gellynck X. A comparison of two low-calorie sweeteners and sugar in dark chocolate on sensory attributes and emotional conceptualisations. Int J Food Sci Nutr. 2018;69(3):344–57.
73. Torrico DD, Tam J, Fuentes S, Viejo CG, Dunshea FR. D-tagatose as a sucrose substitute and its effect on the physico-chemical properties and acceptability of strawberry-flavored yogurt. Foods. 2019;8(7):256.
74. Bell L. Tagatose stability in beverages as impacted by composition and thermal processing. In: Preedy V, editor. Processing and impact on active components in food. Amsterdam: Elsevier Inc.; 2015. p. 605–12.
75. Acevedo W, Capitaine C, Rodríguez R, Araya-Durán I, González-Nilo F, Pérez-Correa JR, et al. Selecting optimal mixtures of natural sweeteners for carbonated soft drinks through multi-objective decision modeling and sensory validation. J Sens Stud. 2018;33(6):1–9.
76. Rubio-Arraez S, Benavent C, Ortolá MD, Castelló ML. Influence of low glycaemic index sweeteners on antioxidant, sensory, mechanical, and physicochemical properties of a watermelon jelly. J Food Qual. 2018;2018:8412017.
77. Son YJ, Choi SY, Yoo KM, Lee KW, Lee SM, Hwang IK, et al. Anti-blooming effect of maltitol and tagatose as sugar substitutes for chocolate making. LWT Food Sci Technol. 2018;88:87–94.
78. Rojo-Poveda O, Barbosa-Pereira L, Orden D, Stévigny C, Zeppa G, Bertolino M. Physical properties and consumer evaluation of cocoa bean shell-functionalized biscuits adapted for diabetic consumers by the replacement of sucrose with tagatose. Foods. 2020;9(6):814.
79. Skytte UP. Tagatose in ready to eat cereals. American Association of Cereal Chemist, Inc. 2002;47(2):224–7.
80. Yang H-I, Ameer K, Eun J-B. Effects of different stevia-to-onion ratios and heating temperatures on physicochemical and sensory attributes of onion-stevia hot water extracts. Food Sci Technol. 2022;42:1–11.
81. Anker CCB, Rafiq S, Jeppesen PB. Effect of steviol glycosides on human health with emphasis on type 2 diabetic biomarkers: a systematic review and meta-analysis of randomized controlled trials. Nutrients. 2019;11(9):1965.
82. Singh SD, Rao GP. Stevia: the herbal sugar of 21st century. Sugar Tech. 2005;7(1):17–24.
83. Ameer K, Bae SW, Jo Y, Lee HG, Ameer A, Kwon JH. Optimization of microwave-assisted extraction of total extract, stevioside and rebaudioside-a from Stevia rebaudiana (Bertoni) leaves, using response surface methodology (RSM) and artificial neural network (ANN) modelling. Food Chem. 2017;229:198–207.

84. Bursać Kovačević D, Barba FJ, Granato D, Galanakis CM, Herceg Z, Dragović-Uzelac V, et al. Pressurized hot water extraction (PHWE) for the green recovery of bioactive compounds and steviol glycosides from Stevia rebaudiana Bertoni leaves. Food Chem. 2018;254:150–7.
85. Yu H, Yang G, Sato M, Yamaguchi T, Nakano T, Xi Y. Antioxidant activities of aqueous extract from Stevia rebaudiana stem waste to inhibit fish oil oxidation and identification of its phenolic compounds. Food Chem. 2017;232:379–86.
86. Koubaa M, Roselló-Soto E, Šic Žlabur J, Režek Jambrak A, Brnčić M, Grimi N, et al. Current and new insights in the sustainable and green recovery of nutritionally valuable compounds from Stevia rebaudiana Bertoni. J Agric Food Chem. 2015;63(31):6835–46.
87. Debnath M. Clonal propagation and antimicrobial activity of an endemic medicinal plant Stevia rebaudiana. J Med Plant Res. 2008;2(2):45–51.
88. Brahmachari G, Mandal LC, Roy R, Mondal S, Brahmachari AK. Stevioside and related compounds – molecules of pharmaceutical promise: a critical overview. Arch Pharm. 2011;344(1):5–19.
89. Brandle JE, Starratt AN, Gijzen M. Stevia rebaudiana: its agricultural, biological, and chemical properties. Can J Plant Sci. 1998;78(4):527–36.
90. Carakostas MC, Curry LL, Boileau AC, Brusick DJ. Overview: the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. Food Chem Toxicol. 2008;46(7 Suppl):1–10.
91. Puri M, Sharma D, Barrow CJ, Tiwary AK. Optimisation of novel method for the extraction of steviosides from Stevia rebaudiana leaves. Food Chem. 2012;132(3):1113–20.
92. Azarpazhooh E, Rashidi H, Sharayei P, Behmadi H, Ramaswamy HS. Effect of flaxseed-mucilage and Stevia on physico-chemical, antioxidant and sensorial properties of formulated cocoa milk. Food Hydrocoll Health. 2021;1:100017.
93. Bender C, Graziano S, Zimmermann BF. Study of Stevia rebaudiana Bertoni antioxidant activities and cellular properties. Int J Food Sci Nutr. 2015;66(5):553–8.
94. Lemus-Mondaca R, Vega-Gálvez A, Rojas P, Stucken K, Delporte C, Valenzuela-Barra G, et al. Antioxidant, antimicrobial and anti-inflammatory potential of Stevia rebaudiana leaves: effect of different drying methods. J Appl Res Med Aromat Plants. 2018;11:37–46.
95. Ntalli N, Kasiotis KM, Baira E, Stamatis CL, Machera K. Nematicidal activity of Stevia rebaudiana (Bertoni) assisted by phytochemical analysis. Toxins. 2020;12(5):1–17.
96. Takahashi K, Matsuda M, Ohashi K, Taniguchi K, Nakagomi O, Abe Y, et al. Analysis of anti-rotavirus activity of extract from Stevia rebaudiana. Antivir Res. 2001;49(1):15–24.
97. Jeppesen PB, Gregersen S, Poulsen CR, Hermansen K. Stevioside acts directly on pancreatic β cells to secrete insulin: actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K PLUS_SPI -channel activity. Metab Clin Exp. 2000;49(2):208–14.
98. Momtazi-Borojeni AA, Esmaeili S-A, Abdollahi E, Sahebkar A. A review on the pharmacology and toxicology of steviol glycosides extracted from Stevia rebaudiana. Curr Pharm Des. 2016;23(11):1616–22.
99. Chatsudthipong V, Muanprasat C. Stevioside and related compounds: therapeutic benefits beyond sweetness. Pharmacol Ther. 2009;121(1):41–54.
100. Jyoti J, Kaur M, Mishra V, Mittal A. Sweet future of stevia: a magical sweetener. Asian J Pharm Clin Res. 2018;11(2):36–42.
101. Goyal SK, Samsher, Goyal RK. Stevia (Stevia rebaudiana) a bio-sweetener: a review. Int J Food Sci Nutr. 2010;61(1):1–10.
102. Serio L. La Stevia rebaudiana, une alternative au sucre. Phytothérapie. 2010;8(1):26–32.
103. Abou-Arab EA, Abou-Arab A, Abu-Salem F. Physico-chemical assessment of natural sweeteners steviosides produced from Stevia rebaudiana bertoni plant. Afr J Food Sci. 2010;4(5):270–81.
104. Lemus-Mondaca R, Vega-Gálvez A, Zura-Bravo L, Kong AH. Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: a comprehensive review on the biochemical, nutritional and functional aspects. Food Chem. 2012;132(3):1121–32.

105. Kaushik R, Narayanan P, Vasudevan V, Muthukumaran G, Antony U. Nutrient composition of cultivated stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts. J Food Sci Technol. 2010;47(1):27–33.
106. Gaweł-Bęben K, Bujak T, Nizioł-Łukaszewska Z, Antosiewicz B, Jakubczyk A, Karaś M, et al. Stevia rebaudiana Bert. leaf extracts as a multifunctional source of natural antioxidants. Molecules. 2015;20(4):5468–86.
107. Ruiz-Ruiz JC, Moguel-Ordoñez YB, Segura-Campos MR. Biological activity of Stevia rebaudiana Bertoni and their relationship to health. Crit Rev Food Sci Nutr. 2017;57(12):2680–90.
108. Žlabur JŠ, Dobričević N, Brnčić M, Barba FJ, Lorenzo JM, Franco D, et al. Evaluation of the behavior of phenolic compounds and steviol glycosides of sonicated strawberry juice sweetened with stevia (Stevia rebaudiana Bertoni). Molecules. 2019;24(7):1202.
109. de Carvalho MW, Arriola NDA, Pinto SS, Verruck S, Fritzen-Freire CB, Prudêncio ES, et al. Stevia-fortified yoghurt: stability, antioxidant activity and in vitro digestion behaviour. Int J Dairy Technol. 2019;72(1):57–64.
110. Bukolt KF, Ramirez N, Saenz A, Mirza K, Bhaduri S, Navder K. Effect of low glycemic index stevia-benefiber sweetener on the physical, textural and sensory qualities of oatmeal raisin cookies. J Food Process Technol. 2019;10(8):804.
111. Rodriguez Furlán LT, Campderrós ME. The combined effects of Stevia and sucralose as sugar substitute and inulin as fat mimetic on the physicochemical properties of sugar-free reduced-fat dairy dessert. Int J Gastron Food Sci. 2017;10:16–23.
112. Wan ZL, Wang JM, Wang LY, Yang XQ, Yuan Y. Enhanced physical and oxidative stabilities of soy protein-based emulsions by incorporation of a water-soluble stevioside-resveratrol complex. J Agric Food Chem. 2013;61(18):4433–40.
113. López V, Pérez S, Vinuesa A, Zorzetto C, Abian O. Stevia rebaudiana ethanolic extract exerts better antioxidant properties and antiproliferative effects in tumour cells than its diterpene glycoside stevioside. Food Funct. 2016;7(4):2107–13.
114. Chen J, Xia Y, Sui X, Peng Q, Zhang T, Li J, et al. Steviol, a natural product inhibits proliferation of the gastrointestinal cancer cells intensively. Oncotarget. 2018;9(41):26299–308.
115. Boonkaewwan C, Ao M, Toskulkao C, Rao MC. Specific immunomodulatory and secretory activities of stevioside and steviol in intestinal cells. J Agric Food Chem. 2008;56(10):3777–84.
116. Gao J, Brennan MA, Mason SL, Brennan CS. Effect of sugar replacement with stevianna and inulin on the texture and predictive glycaemic response of muffins. Int J Food Sci Technol. 2016;51(9):1979–87.
117. Graebin CS. The pharmacological activities of glycyrrhizinic acid ("glycyrrhizin") and glycyrrhetinic acid. In: Reference series in phytochemistry. Cham: Springer International Publishing; 2018. p. 245–61.
118. Matsuoka K, Miyajima R, Ishida Y, Karasawa S, Yoshimura T. Aggregate formation of glycyrrhizic acid. Colloids Surf A Physicochem Eng Asp. 2016;500:112–7.
119. Schmid C, Brockhoff A, Shoshan-Galeczki YB, Kranz M, Stark TD, Erkaya R, et al. Comprehensive structure-activity-relationship studies of sensory active compounds in licorice (Glycyrrhiza glabra). Food Chem. 2021;364:130420.
120. Zhang Q, Gao B, Xiao Y, Yang H, Wang Y, Du L, et al. Purification and characterization of a novel β-glucuronidase precisely converts glycyrrhizin to glycyrrhetinic acid 3-O-mono-β-D--glucuronide from plant endophytic Chaetomium globosum DX-THS3. Int J Biol Macromol. 2020;159:782–92.
121. Pandey DK, Ayangla NW. Biotechnological aspects of the production of natural sweetener glycyrrhizin from Glycyrrhiza sp. Phytochem Rev. 2018;17(2):397–430.
122. Wang HL, Li YX, Niu YT, Zheng J, Wu J, Shi GJ, et al. Observing anti-inflammatory and anti-nociceptive activities of glycyrrhizin through regulating COX-2 and pro-inflammatory cytokines expressions in mice. Inflammation. 2015;38(6):2269–78.
123. Michaelis M, Geiler J, Naczk P, Sithisarn P, Leutz A, Doerr HW, et al. Glycyrrhizin exerts antioxidative effects in H5N1 influenza A virus-infected cells and inhibits virus replication and pro-inflammatory gene expression. PLoS One. 2011;6(5):e19705.

124. Duan E, Zhang B, Liang X, Jing H, Liu C, Zhang F, et al. Effects of glycyrrhizin on the growth cycle and ATPase activity of PRRSV-2-infected MARC-145 cells. Res Vet Sci. 2021;138:30–8.
125. Huang YC, Kuo CL, Lu KW, Lin JJ, Yang JL, Wu RSC, et al. 18α-Glycyrrhetinic acid induces apoptosis of HL-60 human leukemia cells through caspases- and mitochondria-dependent signaling pathways. Molecules. 2016;21(7):872.
126. Domitrović R, Potočnjak I. A comprehensive overview of hepatoprotective natural compounds: mechanism of action and clinical perspectives. Arch Toxicol. 2016;90(1):39–79.
127. Han JB, Wu Y, Wang S, Yi L, Qiu R, Zhou H, et al. Chemical constituents and chemotaxonomic study of Glycyrrhiza glabra L. Biochem Syst Ecol. 2020;92:104130.
128. Isbrucker RA, Burdock GA. Risk and safety assessment on the consumption of Licorice root (Glycyrrhiza sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. Regul Toxicol Pharmacol. 2006;46(3):167–92.
129. Gomaa AA, Abdel-Wadood YA. The potential of glycyrrhizin and licorice extract in combating COVID-19 and associated conditions. Phytomed Plus. 2021;1:100043.
130. Nassiri Asl M, Hosseinzadeh H. Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytother Res. 2008;22(6):709–24.
131. Böttcher S, Drusch S. Saponins – self-assembly and behavior at aqueous interfaces. Adv Colloid Interf Sci. 2017;243:105–13.
132. Dargel C, Hannappel Y, Hellweg T. Heating-induced DMPC/glycyrrhizin bicelle-to-vesicle transition: a X-ray contrast variation study. Biophys J. 2020;118(10):2411–25.
133. Hosseini MS, Ebrahimi M, Abadía J, Kadkhodaei S, Amirian R. Growth, phytochemical parameters and glycyrrhizin production in licorice (Glycyrrhiza glabra L.) grown in the field with saline water irrigation. Ind Crop Prod. 2022;177:114444.
134. Wang ZY, Nixon DW. Licorice and cancer. Nutr Cancer. 2001;39(1):1–11.
135. Ruschitzka F, Quaschning T, Noll G, DeGottardi A, Rossier MF, Enseleit F, et al. Endothelin 1 type A receptor antagonism prevents vascular dysfunction and hypertension induced by 11β-hydroxysteroid dehydrogenase inhibition: role of nitric oxide. Circulation. 2001;103(25):3129–35.
136. Bailly C, Vergoten G. Glycyrrhizin: an alternative drug for the treatment of COVID-19 infection and the associated respiratory syndrome? Pharmacol Ther. 2020;214:107618.
137. Alagawany M, Elnesr SS, Farag MR. Use of liquorice (Glycyrrhiza glabra) in poultry nutrition: global impacts on performance, carcass and meat quality. Worlds Poult Sci J. 2019;75(2):293–303.
138. Račková L, Jančinová V, Petríková M, Drábiková K, Nosáľ R, Stefek M, et al. Mechanism of anti-inflammatory action of liquorice extract and glycyrrhizin. Nat Prod Res. 2007;21(14):1234–41.
139. Vorobyova VI, Skiba MI, Shakun AS, Nahirniak SV. Relationship between the inhibition and antioxidant properties of the plant and biomass wastes extracts – a review. Int J Corros Scale Inhib. 2019;8(2):150–78.
140. Hayashi H, Sudo H. Economic importance of licorice. Plant Biotechnol. 2009;26(1):101–4.
141. I--Food C, Services H. CFR – Code of Federal Regulations Title 21 CFR – Code of Federal Regulations Title 21 Tariq Al-Jallad CFR – Code of Federal Regulations Title 21 Tariq Al-Jallad. US Food & Drug Administration. 2014;(d):5–6. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm.
142. Qiu X, Chen S, Liu G, Yang Q. Quality enhancement in the Japanese sea bass (Lateolabrax japonicas) fillets stored at 4 °C by chitosan coating incorporated with citric acid or licorice extract. Food Chem. 2014;162:156–60.
143. Jiang J, Zhang X, True AD, Zhou L, Xiong YL. Inhibition of lipid oxidation and rancidity in precooked pork patties by radical-scavenging licorice (Glycyrrhiza glabra) extract. J Food Sci. 2013;78(11):C1686–94.
144. Zhang H, Kong B, Xiong YL, Sun X. Antimicrobial activities of spice extracts against pathogenic and spoilage bacteria in modified atmosphere packaged fresh pork and vacuum packaged ham slices stored at 4 °C. Meat Sci. 2009;81(4):686–92.

145. Al-Turki AI, El-Ziney MG, Abdel-Salam AM. Chemical and anti-bacterial characterization of aqueous extracts of oregano, marjoram, sage and licorice and their application in milk and labneh. J Food Agric Environ. 2008;6(1):39–44.
146. Mocanu G-D, Rotaru G, Botez E, Gîtin L, Andronoiu D-G, Nistor O, et al. Sensory evaluation and rheological behavior of probiotic dairy products with Rosa canina L. and Glycyrriza glabra L. extracts. Innov Rom Food Biotechnol. 2009;4:32–9.
147. Maghamian N, Goli M, Najarian A. Ultrasound-assisted preparation of double nano-emulsions loaded with glycyrrhizic acid in the internal aqueous phase and skim milk as the external aqueous phase. LWT. 2021;141:110850.
148. Azami T, Niakousari M, Hashemi SMB, Torri L. A three-step sensory-based approach to maximize consumer acceptability for new low-sugar licorice-chocolate-flavored milk drink. LWT. 2018;91:375–81.
149. Aly AM, Al-Alousi L, Salem HA. Licorice: a possible anti-inflammatory and anti-ulcer drug. AAPS PharmSciTech. 2005;6(1):E74.
150. Sidhu P, Shankargouda S, Rath A, Hesarghatta Ramamurthy P, Fernandes B, Kumar SA. Therapeutic benefits of liquorice in dentistry. J Ayurveda Integr Med. 2020;11:82–8.
151. Asha MK, Debraj D, Dethe S, Bhaskar A, Muruganantham N, Deepak M. Effect of flavonoid-rich extract of Glycyrrhiza glabra on gut-friendly microorganisms, commercial probiotic preparations, and digestive enzymes. J Diet Suppl. 2017;14(3):323–33.
152. Han Y, Pang X, Zhang X, Han R, Liang Z. Resource sustainability and challenges: status and competitiveness of international trade in licorice extracts under the Belt and Road Initiative. Glob Ecol Conserv. 2022;34:e02014.
153. van der Wel H, Loeve K. Isolation and characterization of thaumatin I and II, the sweet-tasting proteins from Thaumatococcus daniellii Benth. Eur J Biochem. 1972;31(2):221–5.
154. Liu JJ, Sturrock R, Ekramoddoullah AKM. The superfamily of thaumatin-like proteins: its origin, evolution, and expression towards biological function. Plant Cell Rep. 2010;29(5):419–36.
155. Ming D, Hellekant G. Brazzein, a new high-potency thermostable sweet protein from Pentadiplandra brazzeana B. FEBS Lett. 1994;355(1):106–8.
156. Faus I. Recent developments in the characterization and biotechnological production of sweet-tasting proteins. Appl Microbiol Biotechnol. 2000;53(2):145–51.
157. Yamashita H, Theerasilp S, Aiuchi T, Nakaya K, Nakamura Y, Kurihara Y. Purification and complete amino acid sequence of a new type of sweet protein with taste-modifying activity, curculin. J Biol Chem. 1990;265(26):15770–5.
158. Morris JA, Martenson R, Deibler G, Cagan RH. Characterization of monellin, a protein that tastes sweet. J Biol Chem. 1973;248(2):534–9.
159. Joseph JA, Akkermans S, Nimmegeers P, Van Impe JFM. Bioproduction of the recombinant SWEET protein thaumatin: current state of the art and perspectives. Front Microbiol. 2019;10(APR):695.
160. de Jesús-Pires C, Ferreira-Neto JRC, Pacifico Bezerra-Neto J, Kido EA, de Oliveira Silva RL, Pandolfi V, et al. Plant thaumatin-like proteins: function, evolution and biotechnological applications. Curr Protein Pept Sci. 2019;21(1):36–51.
161. Science of Thaumatin. Available from: http://www.thaumatin.com/science-of-thaumatin.
162. Pereira CTM, Pereira DM, de Medeiros AC, Hiramatsu EY, Ventura MB, Bolini HMA. Skyr yogurt with mango pulp, fructooligosaccharide and natural sweeteners: physical aspects and drivers of liking. LWT. 2021;150(April):112054.
163. Firsov A, Shaloiko L, Kozlov O, Vainstein A, Dolgov S. Tomatoes expressing thaumatin II retain their sweet taste after salting and pickling processing. J Sci Food Agric. 2021;101(12):5286–9.
164. De Souza VR, Pinheiro ACM, Carneiro JDDS, Pinto SM, Abreu LR, Menezes CC. Analysis of various sweeteners in petit suisse cheese: determination of the ideal and equivalent sweetness. J Sens Stud. 2011;26(5):339–45.

Chapter 6
Vegetal and Microbial Sources of Natural Additives and Their Food Applications

Andrea Vásquez-García, Sandra P. Betancourt-Botero, and Liliana Londoño-Hernandez

6.1 Introduction

According to the Food and Drug Administration (FDA) [1], food additives (FAs) are defined as any added substance that directly or indirectly affects the characteristics of a food. This substance can be incorporated at any stage: processing, packaging, transport, or storage. According to the World Health Organization (WHO), FAs are compounds used to improve or preserve the organoleptic properties of foods, including their texture, flavor, and odor, as well as to prolong their shelf life while maintaining their quality and safety. CODEX STAN 192-1995 regulates the use of additives in the industry worldwide according to the type of food, the functionality, and the source of the additive. In this sense, additives can be of natural or synthetic origin and their functionalities vary between flavor enhancers, sweeteners, colorants, emulsifiers, anti-caking agents, antifoaming agents, among others (Fig. 6.1) [2].

Due to their stability, the most used additives in the industry are of synthetic origin; however, in recent years, due to changes in consumption trends and the proven toxicity of some of these substances used, there has been an increase in research aimed at obtaining natural food additives (NFAs) of different origin, mainly vegetable and microbial, for their application in the food industry. The process of obtaining NFAs may vary according to the type of compound to be produced. However, at a general level, it can follow the following stages: pre-treatment of the raw material, extraction, concentration, purification, and product formation [3].

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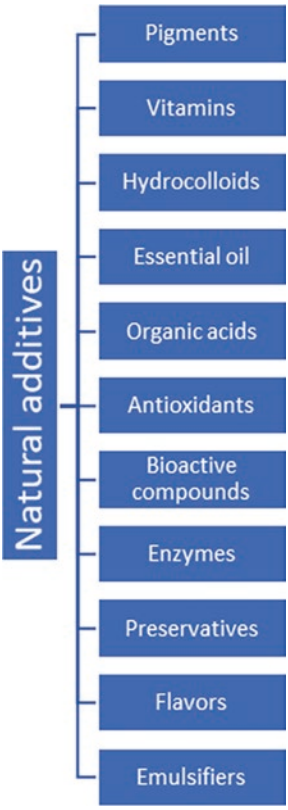

Fig. 6.1 Applications of natural additives in the food industry

Extraction is one of the operations that has been most studied due to its economic implications [3].

Extraction is a unitary operation that allows the removal of a certain compound from a matrix, through several techniques and methods. The separation methodologies traditionally used are solid-liquid extraction, which allows the recovery of a solute (metabolite or bioactive compound) from a solid matrix using a solvent that has an affinity with the compound to be recovered [4]. Although this type of extraction is a simple method, it has several disadvantages, mainly the long processing time, which results in high consumption of solvent and energy, the contamination generated using organic solvents and the low efficiency of the process, which has been considered inefficient [3]. For this reason, in recent years, process engineering has been studying emerging technologies that allow ingredients to be obtained in a sustainable and profitable manner [5]. Among these technologies are high voltage electrical discharges, pulsed electric fields, microwaves, ultrasound, supercritical and subcritical fluids, pressurized liquid extraction, aqueous biphasic extraction, and membrane separation, which have better energy efficiency, use lower extraction temperatures. Furthermore, these emerging technologies have better yields, as well as greater selectivity in the compounds obtained, being considered clean or green technologies [6].

Once the additives are obtained, they can be used in different industries. In the food industry, their use has increased due to changes in consumer habits who demand healthier products. This has led to a growing demand in the additives market in recent years. According to the report by Global Market Insights [7], in 2017 the food additive market exceeded 55 billion dollars and it is expected that by 2024 there will be a consumption of more than 385 million tons. However, this trend may be greater if one takes into account that with the situation resulting from the spread of the COVID-19 virus, consumers tend to eliminate any additives that are not of natural origin from their diet. Therefore, the potential use of these FAs is focused on the development of functional foods aimed at improving consumer health and active packaging to preserve and extend the shelf life of products. Taking into account the above, this chapter aimed to review the main food additives obtained from vegetal and microbial sources, their production process, and the main applications in the food industry.

6.2 Natural Additives Isolated From Vegetal and Microbial Sources

6.2.1 Hydrocolloids

Natural food hydrocolloids (proteins and polysaccharides) [8] have been intensively researched for their application in health improvement or disease prevention through dietary therapy [9]. In foods, these are used for thickening, gelling, fat-replacing, and film-forming [10, 11]. Hydrocolloids of plant origin can be divided into exudates from plants such as acacia gum, chicle gum, glucomannan, gum arabic, gum ghatti, gum tragacanth, inulin, konjac, and pectin. The other group is made up of gums derived from seeds such as amylase, basil seed gum, cassia seed gum, cellulose, fenugreek gum, guar gum, lesquerella fender gum, locust bean gum, karaya gum, konjac, mesquite seed gum, oat gum, rye gum, psyllium, starches, and tamarind gum. The last group is made up of hydrocolloids from seaweed: agar-agar, alginic acid, carrageenan, fucoidan, furcellaran, laminarin, red alga xylan, sodium alginate, and ulvan [12]. Marine macroalgae, particularly brown algae (phylum Ochrophyta or Heterokontophyta) and edible red algae (phylum Rhodophyta) are important sources of unique carbohydrate-based hydrocolloids [13], agar, alginate, and carrageenan are carbohydrate hydrocolloids derived from these algae [14].

Hydrocolloids of microbial origin (fermentation), e.g. baker's yeast glycan,dextran, curdlan, gellan gum, levan, pullulan, spruce gum, scleroglucan, tara gum, welan gum, and xanthan gum [12]. Some bacteria of the genus Pseudomonas and Azotobacter can also produce alginate, but this bacterial compound is acetylated to 2-OH and / or 3-OH and is not used commercially [14]. Another example is the production of xanthan gum product of fermentation by bacteria called as *Xanthomonas campestris* [15]. It is one of the most widely used industrial gum with

an increase in demand and production by 5–10% per year [16]. In the same way, chitin and chitosan extractions have been identified from eight different species of fungi such as *Lentinula edodes* [17], *Pleurotus sajor-caju* [18], *Agaricus bisporus* [19], *Auricula-judae* [20], *Trametes versicolor* [21], *Armillaria mellea* [20], *Pleurotus ostreatus* [22] and *Pleurotus eryngii* [20].

6.2.2 Pigments

Pigments are widely employed because the color is one of the most impressive attributes of foodstuff, cosmetics, medicines, and others, with direct influence on a direct influence on consumer preference, selection, desire, and purchase [23]. In the food industry, several companies are developing foods that contain microalgae or the compounds derived from microalgae. For example, the Mars Wrigley Confectionery company in Ireland, which produces the famous M&M chocolates, uses spirulina as a natural colorant in the process of making these sweets [24]. Among the photosynthetic cyanobacteria, *Arthrospira platensis* stands out for being considered a good nutritional supplement and food additive [25]. This blue-green cyanobacterium is an excellent source of phycobiliproteins (phycoerythrin and phycocyanin) [26]. Phycobiliproteins are complexes of brightly colored proteins and pigments in cyanobacteria (blue-green algae) [27], *rhodophyta* (red algae), *cryptomonads* (or cryptophytes) and *cyanella*. According to the protein structure, there are three major classes of phycobiliproteins consisting of c-phycocyanin, allophycocyanin and phycoerythrin [28]. On the other hand, Anabaena 7120 has been widely studied for being recently recognized as an important source of carotenoids [29]. Some unconventional pigment-producing microalgae (such as carotenoids and phycobiliproteins) are *Chlamydomonas sp*., *Muriellopsis sp*., *Scenedesmus allegiances*, *Tetraselmis sp*. [30], among others.

Several microorganisms, such as *Microbacterium oxydans*, *Chryseobacterium rhizoplanae* JM-534T, *Flavobacterium maris* KMM 9535T, *Chryseobacterium zeace* JM-1085T, *Chryseobacterium arachidis* 91A-593T, *Chryseobacterium geocarposphaerae* 91A-561T, *Rubritalea squalenifaciens sp.*, *Chryseobacterium acrtocarpi* CECT 8497, *Flavobacterium tilapiae* Ruye-71T, *Monascus sp., Staphylococcus kloosii*, *Exiguobacterium aurantiacum*, *Exiguobacterium profundum, Alternaria sp*., *Eurotium rubrum*, *Pseudomonas argentinensis* CH01T, and *Rhodotorula glutinis* have been used in the production of yellowish-orange pigments [31]. Similarly, natural blue pigments are produced by microbial sources, the most studied being those produced by the cyanobacterium *Arthrospira platensis*, eukaryotic algae, cryptophytes and rhodophytes. Finally, *Arthrospira platensis* is responsible for the production of phycocyanin, one of the most important blue compounds approved as safe in the food industry [32].

6.2.3 Antioxidants

Antioxidants can eliminate free radicals and other reactive oxygen and nitrogen species, and these reactive species contribute to most chronic diseases [33]. The cyanobacterium *Arthrospira Platensis* is a source of potent antioxidants, such as carotenoids, polyphenols and phycobiliproteins [34]. High contents of phenolic compounds and flavonoids have been studied in four different species of *Trentepohlia* (*T. abietina, T. arborum, T. diffracta, and T. umbrina*), due to the content of carotenoids, demonstrating the antioxidant activity of these compounds [35]. Spirulina is also well-known for its antioxidant compounds, like phycocyanin and vitamin E [36].

Actinobacteria produce a variety of bioactive compounds of interest to the food industry, including antioxidants. Among the antioxidant compounds of various microorganisms are those produced by *Aspergillus repens, Aspergillus terreus, Aspergillus Versicolor, Cephalosporium sp., Microsphaeropsisolivacea, Nocardiopsis alba, Paecilomyces carneus, Penicilliumparaherquei, Pestalotiopsismicrospora, Streptomyces sp., Streptomyces nitrosporeus, Streptomyces prunicolor* and *Streptomyces tolurosus* [37, 38].

The production of exopolysaccharides can occur because the bacteria present in probiotics can colonize the human gastrointestinal tract, these compounds have an important antioxidant activity [39]. In a study conducted with lactic acid bacteria such as *Lactobacillus plantarum* and *Lactobacillus paracasei subsp. paracasei* produced sufficient exopolysaccharides, which demonstrated high antioxidant activity, such as DPPH free radical scavenging activity, inhibition of linoleic acid peroxidation, chelation of ferrous ions, and reducing power [40].

Natural antioxidants like ascorbic acid (vitamin C) can be found in citrus fruits and tomatoes [41]; alpha-tocopherol (vitamin E) is found in foods such as wheat germ, cereals, broccoli, Brussels sprouts, cauliflower, safflower oil, sunflower oil, almonds, and hazelnuts; beta carotene in kale, spinach, tomatoes, carrots, sweet potatoes, papayas; lycopene in tomatoes, watermelon, pink grapefruit, and guava; selenium in Brazil nuts, cereals, and organ meats; flavonoids in potatoes, tomatoes, lettuce, onions and black tea [42].

Current research reports that various parts of fruits contain phenolic compounds with significant antioxidant capacity [43]. Among the sources of natural antioxidants, apple pomace is rich in catechins, hydroxycinnamates, phloretin glycosides, quercetin glycosides and procyanidins, considered phenolic compounds [44]. Similarly, a study carried out with raspberry pomace was used in fruit purees as an enriching agent because it is rich in ellagic acid, ellagitannin and anthocyanin) [45].

6.2.4 Emulsifier

Emulsifiers are a type of FAs that play an essential role in the physicochemical properties of natural and processed foods, affecting their viscosity, texture, and mouthfeel [46]. Soy lecithin is a naturally extracted amphiphilic molecule, which has been used in various forms in the food industry and is mainly composed of phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, and phosphatidic acid [47]. Emulsifiers are broadly used in the food industry, in baked products, chocolate, cooking spray, instant foods, and margarine [48]. Currently, consumers and regulatory institutions are pushing to replace semi-synthetic and synthetic ingredients in the food industry, within these changes lecithin is considered as a phospholipid with great health benefits [49].

Pea protein is a macromolecule similar to soy protein, which also exhibits similar functional properties such as emulsification and the advantage of generating allergies [50].

Another emulsifiers source is Quillaja saponins, which are isolated from the bark of an endemic tree to the Central Zone of Chile. These natural surfactants usually have a complex mixture of different substances, such as amphiphilic compounds that form micelles when dispersed in water and thus can facilitate the formation and stability of oil-in-water emulsions. The dominant amphiphilic compounds in the natural extracts of this tree are saponins. Saponins are amphiphilic because they have hydrophobic regions (e.g., phenolics) and hydrophilic regions (e.g., sugars), distributed within a single molecule [51].

Some microorganisms that produce emulsifying agents are *Pseudomonas aeruginosa* M408, *Serratia rubidaea* SNAU02, *Bacillus subtilis* LSFM-05, *Achromobacter sp.* HZ01, and *Bacillus brevis* [52]. Biosurfactants produced by *Bacillus subtilis* are used as emulsifiers in the production of biscuits [53]. The formulations with bacterial biosurfactant with 0.1% inclusion showed better dough texture properties such as cohesion, adhesiveness, hardness, and elasticity when compared to the cookies produced with commercial emulsifier glycerol monostearate [53].

Biosurfactants can be produced by yeasts, especially those of genera *Candida*, *Pseudozyma*, and *Yarrowia* [54]. In food formulations, the surfactant compounds produced by *Candida utilis* have the potential to be used. For example, a mayonnaise based on sunflower oil was formulated and *C. utilis* and guar gum were used as biosurfactants, generating a stable mayonnaise for 30 days at 4 °C [55].

Fungal polysaccharides, also known as gums, are hydrophilic substances capable of dissolving or dispersing in water and, as a consequence, increasing the viscosity of a system. Some secreted fungal heteroglucan polysaccharides have various biological activities, for example: *Agrocybe cylindracea* - antioxidant, *Diaporthe sp.* - antitumor, *Pleurotus geesteranus* - anti-inflammatory; hepatoprotective; antioxidant, *Lachnum* YM405 - immunoregulatory, *Penicillium* sp. – pinocytic, *Trametes versicolor* – antioxidant; antitumor, and *Lasiodiplodia* sp. – antimicrobial; antioxidant; immunomodulatory [56].

6.2.5 Vitamins

Several vitamins can be isolated from microbial sources. *Arthrospira platensis* is a cyanobacterium that has a unique composition, comprising not only up to 70% protein containing all essential amino acids, but also polysaccharides, vitamin B12, C, and E [34]. In vitamin E, α-tocopherol stands out, a fat-soluble compound that has a significant antioxidant capacity [57]. Species such as *N. oculata*, *Euglena gracilis*, and *Tetraselmis suecica* have reported the presence of vitamin E, the greatest presence being reported by *E. gracilis* [58]. The consumption of other vitamins produced by cyanobacteria presents benefits for the health of consumers, for example vitamin K1 since *Anabaena cylindrica* has been reported that this vitamin helps prevent chronic diseases, such as cancer and coronary heart diseases [24].

Beta-carotene, a fundamental source of vitamin A, has been efficiently obtained through microbial processes, using the fungus *Blakeslea trispora* and the green microalgae *Dunaliella* [59]. Microbial production of astaxanthin, which occupies a small proportion of the market, is based on the chromophyte microalga *Haematococcus pluvialis* and the basidiomycete yeast X. dendrorhous, originally named *Phaffia rhodozyma* [60]. The production of vitamins using genetically modified microorganisms, such as vitamin B12 from *Pseudomonas denitrificans*, vitamin B2 from *B. subtilis* strains, and *A. gossypii*, vitamin C from *Ketogulonicigenium vulgare*, *Bacillus endophyticus*, *Gluconobacter oxydans* and pro-vitamin A from the strains *Blakeslea trispora*, *E. coli* and *S. cerevisiae* [61]. The genetic modification of microorganisms has been used to increase, for example, the production of ergosterol from S. cerevisiae, this compound is a precursor of vitamin D and is obtained by the overexpression of different enzymes involved in the biosynthetic pathway of the microorganism and the use of substrates more economical as a carbon source, in this case, molasse [59].

Regarding the industrial production of vitamins, they show a significant impact by microorganisms, among which the bacterium *Bacillus subtilis* and the *Ashbya gossypii* fungus to produce vitamin B2 [62] the bacterium Pseudomonas denitrificans to produce vitamin B12 and Luconobacter suboxydansa and Ketogulonicigenium vulgarea bacteria for vitamin C production [60].

There are fungi that use methanol and are naturally capable of synthesizing riboflavin from two main precursors: ribulose 5-phosphate and guanosine triphosphate [62]. Fungal species include *Aspergillus terreus*, *A. gossypii*, *Candida boidinii*, *Candida oleophila*, *C. famata*, *Eremothecium ashbyii*, *Hansenula polymorpha*, *Schwanniomyces occidentalis*, *Pichia guilliermondii*, and *Pichia caribbica* [62]. Other fungi such as *Cephalosporium, Fusarium* and *Trichoderma* have also been investigated for their ability to accumulate ergosterol, however, they offer lower production titers of this compound than *S. cerevisiae* [59].

6.2.6 Fatty Acids

Lipids are rich in polyunsaturated fatty acids (PUFA), used in the food industry as FAs because they help modify the fatty acid composition of specific foods, which is why they are in high demand [51]. The incorporation of PUFA into a food can be done in different ways, the first is the direct addition of PUFA or PUFA-producing microorganisms to the food or by using concentrates with a high content of PUFA that give rise to the production of animal products rich in PUFA, such as eggs, meat, among others [63]. Acids such as arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and linolenic acid are found in PUFAs [24]. High production of PUFA has also been detected from some unconventional microalgae, which represent interesting applications for the food industries, within this group of microalgae are *Isochrysis galbana*, *Aurantiochytrium sp., Cadophora fracta, Chraustochytrids, C. cohnii, Nannochloropsis, Nitzschia sp., Parietochloris incise, P. tricornutum* and *Ulkenia* [64]. There are two possible alternatives to fish oils: single-cell microbial oils and vegetable oils from metabolically engineered plant oilseeds [65]. Successful high-level accumulation of ω3-VLCPUFAs through metabolic engineering of oilseeds has been reported in several species, including *Brassica juncea* [61], *Arabidopsis thaliana* [66] and *Camelina sativa* [67].

Most PUFA synthases originate from bacteria such as *Moritella marina*, *Shewanella sp*., *Colwellia sp*., and *Photobacterium sp*. [68]. On the other hand, most of the PUFA synthases originated from eukaryotic microorganisms such as *Aurantiochytrium sp*., *Schizochytrium sp*. and *Thraustochytrium sp.* [69]. Among the fatty acids of the ω-3 family, C20: 5 eicosapentaenoic acid (EPA) and C22: 6 docosahexaenoic /acid (DHA) are the most important species [70]. EPA is produced by bacteria such as *Shewanella putrefaciens*, *Alteromonas putrefaciens*, *Shewanella electrodiphila* L-proline, and *Photobacterium. Colwellia psychrerythraea* produces DHA. Regardiing the DHA-producing fungi *Thraustochytrium aureum*, *Aurantiochytrium limacinum*, *Schizochytrium limacinum*, and *Schizochytrium* sp. In the family of EPAs produced by fungi we can find species such as *Mortierella alpina* 1S-4, *Pythium irregulare*, *Pythium irregulare*, and *Candida guilliermondii* [71].

6.2.7 Organic Acids

Organic acids are a group of major chemicals that have been frequently employed in some industries, such as food, pharmaceutical, cosmetics, detergent, polymer, and textile [72]. In lactic acid bacteria such as *Lactobacillus sp*. and *Lactococcus sp*., *Bacillus subtilis*, *Corynebacterium glutamicum*, *Escherichia coli*, yeast, and microalgae production of lactic acid has been established [73]. According to Panda et al. [74], lactic acid was produced by *Lactobacillus casei, Lactobacillus*

delbrueckii, and *Lactobacillus plantarum* using potato peas, peas, sweet corn, mango, orange, and fibrous cassava residue as substrates.

In 1924 it was reported that the yeast *Saccharomyces cerevisiae* produced malic acid as a synthesized microbial product [75]. Since that time, other microorganisms that produce L-malic acid have been discovered, including the species of fungi *Aspergillus, Rhizopus*, and *Ustilago*, which are the most promising natural synthesizing microorganisms of this acid in the industry [76]. Malic acid is an intermediate of the tricarboxylic acid cycle and is typically produced in species of the *Aspergillus* fungus, for example, *A. flavus, A. niger*, and *A. oryzae* [73].

Fumaric acid is produced from malic acid by the activity of the enzyme fumarase, there are fungi that naturally produce the fumaric acid *Rhizopus nigricans* or *R. oryzae* [77]. In the biotechnology area, efforts are being joined to establish processes to produce succinic acid using bacterial strains, such as *E. coli* [78]. This acid has various applications in the food industry, such as addictive, foaming agent, surfactant, among others [79]. Furthermore, this acid can serve as a platform for the synthesis of a variety of other chemical substances [80].

The production of citric acid on an industrial scale is typically carried out using the fungus *A. niger* [81]. This fungus naturally produces an excess of citric acid, the molecular mechanism of accumulation and secretion is not yet fully understood [82]. This organic acid can be produced from various agro-industrial waste such as pineapple peel, apple pomace, banana peel, and pineapple pulp [74].

6.2.8 Aroma and Flavor

The synthesis of natural molecules and aromas through microbiological methods can be divided into de novo synthesis and biotransformation [83]. The first consists of the production of aromatic compounds after metabolizing the cells through the use of simple culture media, carrying out the biotransformation through submerged or solid-state fermentation [84]. In the case of submerged fermentation, various substrates and different microorganisms can be used to carry out this biotransformation, some examples are substrates such as Eugenol, using microorganisms such as *Pseudomonas sp*., *Aspergillus niger*, and *Corynobacterium* strains, the vanilla flavor can be obtained. Using lignin as a substrate for the development of *Pleurotus cornucopia*, *Pleurotus pulmonius*, *Pleurotus floridanus* and *Pleurotus eryngii* to obtain Anisaldehyde that generates flavors such as vanilla and anise. The growth of microorganisms such as *Ischnoderma benzoinum* and *Kluyveromyces lactis* using L-phenylalaline as a substrate produces 2-phenylethanol which is used as a food additive [84].

In the case of solid-state fermentation, using a substrate of cassava bagasse with valine and cassava bagasse with leucine for the growth of the microorganism *Ceratocystis fimbriata*, developing a banana aroma [85]. The pregelatinized rice substrate allows the growth of *Neurospora sp*., generating aromas such as Ethyl acetate, Ethyl caproate, Isoamyl alcohol, 1-Octen-3-ol, and 3-Methyl-1-butanol and

Bacillus subtilis generates pyrazine. *Ceratocystis fimbriata* can grow on various agro-industrial substrates such as amaranth, cassava bagasse, coffee peel, apple pomace and soy, producing fruity aromas, and pineapple aroma [86]. Some investigations have reported that the growth of this microorganism on substrates such as coffee husks supplemented with 20% and 30% glucose generates a pineapple aroma. In the year 2000, the growth of the microorganism *Kluyveromyces marxianus* on palm bran and cassava bagasse substrate, producing fruity aromas, was investigated [86]. Similarly, the biotechnological production of traditional aromas such as concentrated aromas Cheddar and Roquefort type cheese, are obtained by the cultivation of species such as *Micrococcus sp., and Penicillium roqueforti,* respectively [87].

De novo synthesis should be used for the preparation of product mixtures, while biotransformations can carry out single-stage processes [88]. To produce unique aromatic compounds, microorganisms grown in specific and appropriate media are used for their growth and development, due to the complex transformation of molecular bioconversion [87]. Some of the aromas produced are Benzaldehyde, with a sensory description of bitter almonds, the sensation produced by various species of fungi *Agaricus bisporus*, *Agaricus subrefecens*, *Armillaria mellea, Ischnoderma benzoinum, Pleurotus sapidus, Polyporus sp.,* and *Tyromyces sambuceus*. In the case of the anisaldehyde aroma with the sensory perception of vanilla, the anise aroma is produced by fungi such as *Bjerkandera adusta Pleurotus sapidus, Polyporus benzoinus*, and *Trametes suaveolens*. The aroma of methyl benzoate and ethyl benzoate presents a fruity aroma generated by the growth of fungi such as *Agaricus subrefecens, Mycena pura, Phellinus sp.,* and *Polyporus tuberaster* [84].

6.2.9 Enzymes

Microorganisms such as bacteria, yeasts, and fungi are used to produce enzymes that are then used in various food preparations to improve taste and texture and offer enormous economic benefits [89]. In the food industry, the use of enzymes stands out, for example in the saccharification of starch carried out by α-amylase enzymes produced by *Bacillus amyloliquefaciencs, Bacillus licheniformis*, or *Bacillus stearothermophilus* [90]. Other important enzymes are glucoamylases produced mainly by the fungi *Aspergillus niger, Aspergillus awamori*, and *Rhizopus oryzae*, the enzymes produced by the latter fungus are widely used for industrial applications [91]. Acid protease from *Aspergillus usamii* has been used successfully to improve the functional properties of wheat gluten [92]. The β-galactosidase is produced from the yeast *Kluyveromyces lactis* and *Kluyveromyces fragilis* [93]. One advantage of the use of enzymes of microbial origin is that many applications cannot be fulfilled through chemical synthesis, as is the case of the lipase produced by *Candida rugosa* [94]. The commercial phospholipase produced by *Fusarium oxysporum* is marketed by the Danish company Novozymes A / S and its applications include baking [95]. *Bacillus licheniformis* produced a new thermostable esterase, a highly thermotolerant enzyme which was expressed heterologous in *E. coli* [96]. Patel et al. [97]

obtained with the growth of the microorganism *Lasiodiplodia theobromae* the purified lipoxygenase enzyme using different chromatography techniques and achieved the complete characterization of the enzyme. The main species of xylanase-producing fungi are *Aspergillus sp., Fusarium sp.* and *Penicillium sp.* [98].

The fungi *Aspergillus niger*, and *Penicillium glaucum* were identified as the first producers of glucose oxidase, although there are several microorganisms that produce it [89]. Aspergillus niger species is widely used in the food industry to produce glucose oxidase [99]. *Penicillium adametzii* is a fungus widely used for extracellular glucose oxidase production [89]. Laccases are secreted extracellularly by various fungi as a product of their secondary metabolism during fermentation, but their production is limited to a few species of fungi [100]. Fungi from the deuteromycetes, ascomycetes as well as basidiomycetes are known producers of laccase [101]. White rot fungi can produce laccases, among these fungi is *Funalia trogii.* Laccases can also be produced from microbial sources such as *Aspergillus niger*, and *Micrococcus luteus*. Microbial enzymes are generally preferred as sources to produce enzymes due to several advantages, such as rapid growth, easy handling, and genetic modification that allows obtaining the desired enzyme [102].

6.2.10 Preservatives

Food preservation includes many science-based applications through accessible procedures or technologies to stop the spoilage of food products and to extend their shelf life, reassuring customers that a product is free of pathogenic microorganisms [103]. Polyphenols and essential oils of plant origin can be mentioned as examples of natural preservatives [104]. Special attention has been paid to the applications of vegetable essential oils as food preservatives, coming from plant families such as: *Monimiaceae, Lamiaceae, Lamiaceae, Apiaceae, Zingiberaceae Burseraceae, Piperaceae, Asteraceae, Ranunculaceae, Cyperaceae, Lamiaceae, Apiaceae, Rosaceae Cardiopteridaceae, Myrtaceae* and *Apiaceae Lamiaceae* [105]. Essential oils obtained from spices contain active compounds that exhibit great antimicrobial potential and can be used in the food industry, such as 3-phenylprop-2-enal, 5-isopropyl-2-methylphenol, etc [106]. The above-mentioned compounds show antimicrobial activity against *Aspergillus spp., Escherichia coli, Listeria monocytogenes, Shigella sonnei* and *Shigella flexneri* [107].

There are natural antimicrobials for the biopreservation of foods derived from bacterial cell metabolism, among which are organic acids, CO_2, diacetyl, hydrogen peroxides, reuterin, and reutericicline [108]. Furthermore, preservatives can be produced by fungi such as Streptomyces natalensi that produces natamycin [109]. Similarly, there are antimicrobials derived from plants such as the essential oils carvacrol, citral, eugenol, flavonoids, linalol, saponins, thymol, and terpenes from plant material such as buds, bark, flowers, fruits, herbs, leaves, wood, seeds, branches, and roots [108].

6.3 Production Process

Additives for use in food come from various sources and their production and/or extraction processes are equally varied. Table 6.1 summarizes information related to traditional production processes, some studies on novel processes for obtaining these, and both vegetable and microbial sources.

6.3.1 Novel Extraction Processes for Food Additives

In order to increase the extraction yield and improve the quality of the substances of interest in the food industry to be used as FAs, advances have been made through the search for alternative production processes and/or pretreatments that favor the current extraction techniques. In this segment, we will discuss some of these processes such as ultrasound-assisted extraction, the use of supercritical fluids in extraction processes, microwave-assisted extraction, among others.

6.3.1.1 Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction is used in many food processing operations; some of them are emulsifying, drying, extraction, etc. UAE is considered a "green" technology since it is a simple process, with lower consumption of solvents and increased yields. There are two methods used, through ultrasound probe or ultrasonic bath [134], the combination of different methods for food processing with these two technologies provides processes with higher yields in shorter process times.

UAE has been used for the extraction of different substances from a variety of sources like Hibiscus sabdariffa where the temperature, time, and concentration of solvents were evaluated to obtain the best combination of variables, through the measurement of performance and concentration of components, they concluded that the process variables to obtain the best extraction yield were 164 °C, 60% ethanol, and 22 min [135]. Other studies applied UAE for the obtention of pepper seeds oil using n-hexane as a solvent in an ultrasound bath comparing the results with other technologies as pressure-assisted and conventional technologies; in this case, the UAE process variables were 50 °C, 200 W for 50 minutes obtaining an Efficiency extraction (Ee) of 83.52 ± 4.84%, which was not statistically different from the Ee using the other technologies, but the pressure-assisted extraction produced the highest level of unsaturated fatty acids (82.37%) [136]. UAE is also used for the extraction of phenolic compounds from mango peels evaluating the mixture of ethanol, acetone, and hexane [137], Red beet root (*Beta vulgaris* L.) using β-cyclodextrin as a solvent improving betanin extraction [138] or Pomegranate (*Punica granatum* L.) peel using ethyl acetate, ethanol, methanol, 50% aqueous methanol and water as solvents obtaining a processing time shorten by 20 times. This technology was also

Table 6.1 Additive sources, traditional and novel extraction, and production processes

Additive type	Traditional production process	Novel extraction process	Additive source
Hydrocolloid	Agar: Mainly obtained from *Gelidium cartilagineum, Pteroclaia capillacea, Gracilaria vermiculophylla, and Gracilaria confervoides* through washing the dried seaweed with hot water for several hours Xanthan gum: Obtained by carbohydrate fermentation in batch and/or fed batch conditions with *Xanthomonas pelargonii, campestris, malvacearum* and *phaseoli* [110] Gellan gum: biopolymer secreted by *Sphingomonas paucimobilis* [111] Aerobic fermentation of a culture medium (grape pomace as carbon source, molasses, and cheese whey-based medium) [112] Pectins: Use of strong mineral acids under heating to extract pectin from plant tissue (HCL, H_2SO_4)	Agar: Combined ultrasound-assisted hot water extraction procedures [113] Alkali pretreatment, enzyme assisted extraction methods [114] Fermentation with LED irradiation of *X. campestris* [115] Enzyme treatment of carbohydrate sources [114] New strains as newly-isolated Sphingomonasazotifigens GL-1 H_2O_2-induced oxidative stress [116] Organic acids extraction of pectin and xylooligosaccharides [117] electric fields extraction of pectins [118] Ultrasound-assisted dilute acid hydrolysis from orange peels [119]	Plants: acacia gum, chicle gum, glucomannan, gum arabic, gum ghatti, gum tragacanth, inulin, konjac, and pectin Seeds: amylase, basil seed gum, cassia seed gum, cellulose, fenugreek gum, guar gum, Lesquerella fender gum, locust bean gum, karaya gum, konjac, mesquite seed gum, oat gum, rye gum, psyllium, starches, and tamarind gum Seaweed: agar-agar, alginic acid, carrageenan, fucoidan, furcellaran, laminarin, red alga xylan, sodium alginate, and ulvan

(continued)

Table 6.1 (continued)

Additive type	Traditional production process	Novel extraction process	Additive source
Antioxidants	Phenolic compounds: Liquid-liquid extraction or solid-liquid extraction	Phenolic compounds: Pretreatment with cold plasma [120] Ascorbic acid: Somatic fusion of *Aspergillus flavus* and *Aspergillus tamarii* [121]	*Aspergillus repens, Aspergillus terreus, Aspergillus Versicolor, Cephalosporium sp., Microsphaeropsisolivacea, Nocardiopsis alba, Paecilomyces carneus, Penicilliumparaherquei, Pestalotiopsismicrospora, Streptomyces sp., Streptomyces nitrosporeus, Streptomyces prunicolor* and *Streptomyces tolurosus*
Pigments	Carotenoids and Chlorophyll: From microalgae *Dunaliella Salina* is obtained by solvent extraction (hexane/ethanol) [122]	Red and Yellow pigments from fungi: Produced by solid-state fermentation (SSF) with *Monascus sanguineus* NF CC I 24 53 using rice as the carbon source and with solid-liquid extraction [123]. SSF of potato pomace as carbon source with *M. purpureus* solid-liquid extraction (ethanol, methanol) [124]	The production of natural blue pigments, those from bacteria *Streptomyces coelicolor* [125], *Pseudomonas aeruginosa* [126], *Pseudomonas* spp. and *Acinetobacter* spp. [127], *Pseudomonas indigofera*, *Erwinia* spp and *Streptomyces* spp. [128], *Nonomuri*a sp. *Arthrospira platensis.* In the case of fungi, *A. caeruleoporus, A. cristatus, Alabtrellus flettii*, and *A. confluens* [129], *Ceratocystis minor Corticium caeruleum Penicillium herquei* phenalenone, *Gremmeniella abietina Suillus* (Boletus) *variegatus* [32]
Emulsifiers	Mono and diglycerides of fatty acids are obtained using two chemical processes: direct esterification of glycerol and fatty acids and transesterification of glycerol [44]	Mono and diglycerides of fatty acids can be obtained from microalgae [130]	The bioemulsifiers produced by fungi *Candida tropicalis*, *Candida lipolytica* Y-917, *Candida ingens*, *Candida lipolytica* UCP0988, *Candida tropicalis*, *Candida bombicola*, *Candida apicola Candida lipolytica* ATCC 8662, *Penicillium chrysogenum*, *Yarrowia lipolytica* IMUFRJ 50682, *Yarrowia lipolytica* NCIM 3589, *Yarrowia lipolytica* IMUFRJ 50682 *Ustilago maydis*, *Candida sphaerica* UCP0995 *Candida glabrata* and *Pseudomonas aeruginosa* [56]

(continued)

Table 6.1 (continued)

Additive type	Traditional production process	Novel extraction process	Additive source
Vitamins	Vitamin K1 is produced by Chemical synthesis [131] B2 is industrially produced by chemical synthesis and fermentation [62] Vitamin C, B2, D2 are produced by fermentation with bacteria, yeasts or fungi [59]	Vitamin K1 and B12 produced by cyanobacterium *Anabaena cylindrica* [131]	The production of vitamins using genetically modified microorganisms, such as: *Pseudomonas denitrificans*, *B. subtilis* strains, *A. gossypii*, *Ketogulonicigenium vulgare, Bacillus endophyticus, Gluconobacter oxydans*, *Blakeslea trispora, E. coli* and *S. cerevisiae* [61]
Fatty acids		Fermentation process with engineered *E. coli* [132]	*Isochrysis galbana, Aurantiochytrium sp., Cadophora fracta, Chraustochytrids, C. cohnii, Nannochloropsis, Nitzschia* sp., *Parietochloris incise, P. tricornutum* and *Ulkenia*
Organic acids		Produced by Fermentation process with bacteria, yeasts or molds Acetic acid, lactic acid, butyric acid, citric acid, tartaric acid, succinic acid [133]	*Lactobacillus* sp. and *Lactococcus* sp., *Bacillus subtilis, Corynebacterium glutamicum, Escherichia coli,* yeast, and microalgae

used for protein isolates by Wang et al. [139] using an experimental design of BoxBehnken with a maximum extraction of 82.6%. Natural food colorants have been obtained from agro-food products like aracá peels [140] jaboticaba epicarp [141], and Curcuma longa L. [142].

6.3.1.2 Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is a widely used extraction technology that uses carbon dioxide as a solvent to obtain polar substances. In this technology, ethanol is generally used as a co-solvent [143]. These solvents have gas-like properties and low viscosities, which enhances the mass transfer process [144]. Rodríguez-Espana et al. [145] applied SFE to extract lipids and focused especially on the concentration of docosahexaenoic acid (DHA) obtained from *Schizochytrium sp.* The optimum process variables were obtained by using a central compound design 22 and a response surface methodology, concluding that using 46.52 MPa and 76.86 °C the lipid yield extraction was 16.95%. In another research, Kargılı and Aytaç [143] evaluated the effect of pressure and temperature on cannabinoid extraction for

different types of cannabis plants, with and without ethanol as a cosolvent. The authors observed that an increment in the pressure value using the same temperature increased the solubility and consequently the extraction yield from 6.54% to 8.44% for a temperature process of 60 °C and from 6.90% to 9.68% using 40 °C. SFE was also used to obtain a polysaccharide from fallen Ginkgo leaves with an ethanol/water solution as a cosolvent; in this study, the optimized conditions were 90 minutes, 42 MPa, 63 °C, and cosolvent solution at 68% for a maximum extraction yield of 10.13 ± 0.12 g/100 g [146]. On the other hand, to extract caffeine from Yerba Mate Timm do Espirito Santo et al. [147] kept the pressure (300 bar), temperature (60 °C), and particle size as constants. The researchers used a Box-Behnken design to optimize carbon dioxide flux, ethanol flux, and extraction time, obtaining the following optimized process variables: carbon dioxide flux of 950 g/h; 106 g/h ethanol flow rate, and 4.25 h with a change in caffeine concentration from an initial value of 2.1% (g caffeine/g yerba mate) to 0.16 ± 0.06% (g caffeine/g yerba mate).

6.3.1.3 Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction (MAE) uses non-ionizing radiations and frequencies from 300 MHz to 300 GHz. The solvent used in this process can be polar or non-polar, with polar solvents, extractions can be carried out with better absorption of microwave energy [148], but in oil extraction, non-polar solvents exhibit better solubility. Hu et al. [149] compared MAE with the Soxhlet method for the extraction of *Sapindus mukorossi* seed oil, using different concentrations of ethanol and n-hexane. The authors concluded that the amount of n-hexane affected the extraction yield, being that the best result was obtained for a proportion n-hexane:ethanol of 4:1, v/v for Soxhlet method. They also obtained optimized process variables for MAE using a Box-Behnken Design (BBD); and the variables evaluated were microwave power (300–500 W), solvent to material ratio (6–10 mL/g), extraction temperature (60–80 °C) and extraction time (30–50 min) with an optimum value, according to the model of 462.1 W, 8.1 mL/g, 72.3 °C and 42.2 min respectively, with no significant differences on extraction yield compared with Soxhlet method, but a higher process efficiency, lower energy consumption and better quality (lower acid and peroxide index values); it was also noticed that internal structure was affected by MAE. On the other hand, Liu et al. [150] used MAE for the extraction of Proanthocyanidins from *Cinnamomum camphora* optimizing variables as solvent concentration (ethanol), liquid/solid ratio, microwave time, and microwave power obtaining the best results for liquid/solid ratio of 20 mL/g, 77% of ethanol concentration, time of the process of 18 min and microwave power of 530 W, under these conditions, proanthocyanidins showed high values of antioxidant activities that makes the obtained product a promising option as a natural antioxidant [150]. This technology is also used to take advantage of substances of industrial value in agro-industrial by-products, Figueroa et al. [151] optimized the extraction of bioactive polyphenols from avocado peels using a central composite design and concluded that an ethanol concentration of 36%, a solvent to peel ratio of 44 mL/g, and a time

and temperature of 39 minutes and 130 °C, respectively, were the best conditions to recover the phenolic compounds from avocado peels.

6.3.1.4 Enzyme-Assisted Extraction (EAE)

Enzyme-assisted extraction (EAE) is an ecofriendly alternative widely used due to the high selectivity of enzymes that allow obtaining products with high purity and quality. With this in mind, Domínguez-Rodríguez et al. [152] used enzymes for the extraction of non-extractable polyphenols (phenolics and proanthocyanidin), the antioxidant capacity and the ACE inhibition capacity were measured. The authors concluded that the polyphenols obtained by EAE had higher bioactivity than those obtained by alkaline and acid hydrolysis. The extraction of phenolic compounds was also measured on soy flour by Dias de Queirós et al. [153], who evaluate the effect of the enzyme's protease, tannase, and cellulase on bioaccessibility, extraction, and bioconversion. These authors observed a synergistic effect of the tested enzymes and concluded that the use of enzymes improves the extraction processes and the combination of protease, tannase, and cellulase had a synergistic effect. On the other hand, Li et al. [114] combined EAE with ultrasonication and alcali pretreatment to extract agar from *Gelidium sesquipedale* and concluded that Alcalase (proteinase) and Viscozyme (carbohydrase) were the best of the five enzymes studied for agar extraction (Alcalase, Neutrase, Papain, cellulase and Viscozyme). The enzyme-assisted extraction technology was tested on lotus leaves to extract polysaccharides using α-amylase, cellulase, pectinase, and protease finding that only protease had a significant improving effect on yield extraction compared with the traditional hot-water extraction process. The extraction method affected molecular weight distribution, and the polysaccharides obtained were mainly arabinose, galactose, rhamnose, and galacturonic acid [154].

6.3.1.5 Cold Plasma-Assisted Extraction

Plasma is known as the fourth matter state, and it is obtained through the ionization of gas by injecting energy into it. Cold plasma is generated at low temperatures at vacuum or atmospherical conditions, it is used to enhance extraction yields of different food products such as essential oils [155], phenolic compounds [120] and is also used as a pretreatment for drying fruits, reducing their processing time. This reduction can be caused by the formation of irregular microscopic channels formed with the cold plasma treatment [156].

During the extraction of essential oils from fennel seeds and spearmint leaves by hydrodistillation, Rezaei et al. [155] used Dielectric barrier discharge cold plasma technology as a pretreatment obtaining an improvement in extraction yield near 1.8% (v/w) for both essential oils sources, with optimum process variable values according to the Response Surface Methodology (RSM) of 10 min exposure time, and 19 kV applied voltage with air as the input gas. On the other hand, for the

improvement of phenolic compounds extraction by high voltage atmospheric cold plasma (HVACP) were used, with the generation of surfaces with a higher hydrophilic capacity due to the disruption of the structure of tomato pomace, and also different profiles of phenolic compounds were obtained [120].

6.3.1.6 Pulsed Electric Fields (PEF)

Pulsed electric fields (PEF) are studied for microbial inactivation in foods to enlarge their shelf life. Nowadays it has been studied to improve the extraction yield of substances that can be applied to food processing as phycocyanin, proteins, and carbohydrates from *Arthrospira maxima* [157], phenolic compounds such as betaxanthins and betacyanins from cinnamon [158], and betalains from red beetroot [159]. According to the study on cinnamon, the researchers concluded that Pulsed Electric Fields can be used as a pretreatment for extraction processes by increasing cell permeability affecting the yield and characteristics of the products obtained [158]. For PEF-treated spirulina, cell rupture was also noted, causing leakage of substances such as proteins and pigments [157]. During the extraction of naringin from grapefruit peel, an increase in the extraction yield was obtained using PEF as pretreatment (15.8 mg/mL) compared to the product not treated with PEF (13.49 mg/mL). The processing variables to obtain naringin from the white part of the grapefruit peel were from 0 to 50 pulses with intensities from 0 to 10 kV/cm, being observed that as the intensity of the electrical pulse or the number of pulses increased, the tendency obtained was to reach a maximum point with the consequent reduction in the extraction yield [160]. On the other hand, moderate electric fields (MEF) at 60 Hz were used to extract pectin from passion fruit, with a process temperature not higher than 50 °C. In this case, the extraction yield with FEM was lower than the yield of the traditional extraction process [161].

6.4 Food Applications

In recent years, due to the variation in consumption trends and the search for healthy, fresh, and ready-to-eat foods, research on NFAs and their applications in the food industry have increased. Some of the main additives used and their application in the food industry are shown in Fig. 6.2. The applications of additives in food processing are varied, one of the main uses of these additives focuses on increasing the shelf life of products, controlling the appearance of microorganisms that cause deterioration, and the developing of reactions that modify the organoleptic properties of food products.

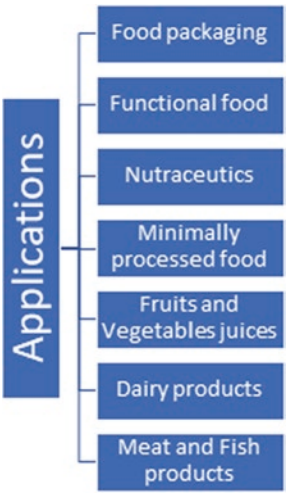

Fig. 6.2 Applications of natural additives in the food industry

6.4.1 Food Packaging

Packaging in the food industry is a fundamental part of the process because it maintains the integrity of the products until they reach the final consumer. Packaging fulfills different functions, mainly maintaining the shelf life of the foods, avoiding contamination and handling during transportation. Nowadays, innovations have led these packages to have additional functions, being considered active or intelligent packaging. In active packaging, different compounds are incorporated to maintain and increase the shelf life of the product, while intelligent packaging can have biosensors or indicators to monitor product conditions [162].

For both active and intelligent packaging development, the use of natural compounds of plant or microbial origin has been increasing. Applications of NFAs in packaging development include increasing shelf life by preventing contamination by microorganisms, dehydration, oxidative rancidity, among others, or giving functionality to products by adding bioactive compounds with biological activity that help improve consumer health [163].

In recent years, several plant-based additives have been investigated to improve the characteristics and properties of biodegradable packaging and films. Among these, Moraczewski et al. [164] evaluated the use of natural extracts of cocoa, coffee, and cinnamon on some properties such as color, transparency, roughness, wettability, coefficient of friction, adhesion strength and gas permeability in polylactide films. The researchers found that in all three cases the films showed improved characteristics, including improved characteristics such as aging predictability. In general, surface roughness increased, wettability improved, and adhesive strength increased. This is possibly due to the concentration and type of polyphenols in each sample, for this study a higher concentration of chlorogenic acid, flavonoids and phenolic acids was found for coffee, cocoa, and cinnamon, respectively.

Another vegetable additive that has been used in the development of films and packaging in recent years has been essential oils. Essential oils are known to have different biological activities, including antimicrobial properties, so their use in packaging can be very useful. Considering this, Vishnu et al. [162] incorporated essential oil from the plant *Plectranthus amboinicus* into films made from chitosan. They had previously determined that the oil had antimicrobial activity against *Staphylococcus aureus, Salmonella typhimurium, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, so they believe that the films can be used to increase the shelf life of foods that present frequent alterations by the microorganisms mentioned.

Like plant-based additives, additives of microbial origin have potential applications for packaging development, either to improve characteristics or to add specific functionalities. Among the microbial additives of interest are colorants, which may replace synthetic colorants derived from petroleum, whose negative effects on the environment have been documented. On this topic, de Oliveira et al. [165] evaluated the production of a yellow-orange-red dye in a stirred tank bioreactor using the fungus *Talaromyces amestolkiae* and its application in a biodegradable film made from cassava. For this case, the researchers established the process conditions (2.0 vvm, 30 °C, and 500 rpm) that would allow obtaining the highest concentration of dye by liquid fermentation. Once the colorant was obtained, they applied it to the film, demonstrating that in addition to improving the appearance of the packaging, it was able to reduce the oxidation of the butter, the product under study, improving its shelf life.

Another microbial compound with potential application in packaging development is exopolysaccharides (EPS). These EPS can be used as additives to improve the structural properties of films or as encapsulating agents for bioactive compounds or probiotic microorganisms that are used in active packaging [166]. In recent years, applications in packaging development of postbiotics, defined as bioactive compounds or substances produced during the metabolism of probiotic microorganisms and released into the environment, have also been evaluated [167]. Although the concept is recent, several research have been carried out on the subject, Kürsad Incili et al. [168] evaluated the postbiotic profile of the microorganism *Pediococcus acidilactici* and the inclusion of the compounds in films developed with chitosan.

6.4.2 Functional Foods

The concept of functional foods first appeared in Japan around 1984 when a new category of foods described as Food for Specific Health Uses (FOSHU) was created, which were defined as “foods containing an ingredient with health functions and officially approved to claim their physiological effects on the human body” [169]. Since this declaration, different institutions have provided definitions of the concept of functional foods. In general, it is considered that these foods should contain vitamins, minerals, or bioactive compounds in adequate concentrations to provide a benefit to human health, beyond basic nutrition. Among some research on

the addition of NFAs to obtain functional foods, there is the one carried out by Yessuf et al. [170] who developed a moringa-based drink with the addition of natural extracts of mango, avocado, lemon, among others, with the objective of reducing problems related to malnutrition. Once the best beverage was selected according to the sensory analysis, a characterization was carried out and it was found that the product is a source of minerals, especially iron, an essential mineral for nutrition. Corsetto et al. [171] evaluated the extraction process of compounds with antioxidant activity from seaweed and their subsequent use in rye snacks. The extracts obtained from brown seaweed *Fucus vesiculosus* present a polyphenol content between 0.26–0.30 g PGE/g and exhibit antioxidant activity. Subsequently, it was determined that the inclusion of 5% of extract to extruded rye snacks allows obtaining a product with functional characteristics.

In recent years, numerous research studies have been oriented to obtain compounds for the development of functional and nutraceutical foods; however, one of the great challenges is to ensure that these compounds remain stable within the production process and even more so during their transit through the digestive system, being released in the appropriate place where they exert their beneficial effect. Additionally, the incorporation of these compounds can alter the organoleptic characteristics of foods or limit the bioavailability of some nutrients. Therefore, the processes of innovation and development of functional foods require the use of technology such as encapsulation to guarantee the protection of functional compounds [172]. The purpose of encapsulation is to protect sensitive compounds by coating them with different materials. Encapsulation can protect compounds from oxygen, high temperatures, damage caused by light or others [173]. In this way, Kumar et al. [174] evaluated the addition of encapsulated squalene, as a natural additive with antioxidant characteristics, to muffins to obtain a functional product. The squalene capsules were added during processing and before baking. Proximal analysis showed that the nutritional characteristics of the muffins improved, as well as the texture. In general, the researchers indicate that encapsulation protects the compounds during processing to obtain ready-to-eat functional foods.

6.4.3 Other Food Products

In general, in the food industry, NFAs are being used to improve the physicochemical and organoleptic characteristics of products in different areas: fruits, cereals, meats, fish, and dairy products, and even to increase their shelf life. In dairy products and derivatives, one of the most frequent problems is lipid oxidation, which causes alterations mainly in flavor as well as the formation of volatile and non-volatile compounds that modify the odor. Likewise, oxidation can cause the modification of vitamins and amino acids causing the loss of functionality of some compounds, mainly proteins, which not only alters the quality of the product but can also affect the consumer's health, which is why the inclusion of NFAs with antioxidant characteristics reduces this type of reactions [175]. NFAs can also help the

nutritional quality of dairy products and derivatives. Alibekov et al. [176] studied the process of cottage cheese fortification with NFAs from ginger, topinambour, and radish. The researchers incorporated the natural extracts during the process, after the formation of the coagulum. Once the cheese was obtained, they performed physicochemical and sensory analyses, finding that the addition of 3–5% of natural extracts favored the presence of minerals and other compounds such as retinol (vitamin A), thiamine (B1), folacin (B9), riboflavin (vitamin B2), tocopherol (vitamin E), and ascorbic acid (vitamin C), obtaining a nutritionally better product.

In the case of fruits, vegetables and their derivatives, the applications of NFAs are mainly oriented to improve the organoleptic characteristics of the product, including texture, flavor, and color. In this case, one of the most widely used additives are hydrocolloids, which are attributed, among others, properties such as stabilizers and texture modifiers. Considering this, Quintana Martínez et al. [177] evaluated the use of hydrocolloids extracted from butternut squash (*Cucurbita moschata*) peels to improve the texture of papaya jam. Initially, they evaluated the process of obtaining the hydrocolloids by varying the pH, and once obtained, it was added to the product, finding that although the rheological characteristics of the product and its color were altered, with the addition of 0.25% of hydrocolloids a product of similar sensory acceptance to the control was achieved. Therefore, the hydrocolloids obtained from butternut squash peels improve the physical stability of the products, increasing their shelf life. Among other studies, El-Saadony et al. [178] evaluated the use of natural extracts to improve the physicochemical and microbial stability and increase the shelf life of cucumber juice, for which they evaluated the use of citric acid, benzoic acid, sodium salts, hydrolyzed pepsin hydrolysate from butternut squash peels, and hydrolyzed pepsin hydrolysate from butternut squash peels, bean pepsin hydrolysate, chicken egg protein isolate, duck egg protein isolate, and duck egg protein isolate, and quail egg protein isolate, finding that bean pepsin hydrolysate exhibits antimicrobial activity against *B. cereus, L. monocytogenes, E. coli,* and *P. aeruginosa*, and cucumber juice with the addition of this hydrolysate maintains antioxidant activity during 6 weeks of storage. Therefore, the researchers propose the use of natural additives to improve the quality and shelf-life stability of fruit juices and beverages. Color is an important aspect in the food industry, intervening in the consumer's decision to buy, so it should remain stable during the shelf life of the product. Vega et al. [179] evaluated the stability of colorants obtained from *Rubus fruticosus* L. and *Morus nigra* L. in fruit drinks, finding that although the content of some compounds such as anthocyanins decreases over time, the color is maintained even after 12 weeks of storage, so the use of natural colorants from microorganisms can be considered an alternative to the use of synthetic colorants in food products.

One of the challenges in the production of bakery products is the fortification with minerals to support the nutrition of different population groups. Rogaska et al. [180] evaluated the bioavailability of iron, zinc, and copper in gluten-free bakery products with the addition of poppy seeds as a natural ingredient compared to synthetic additives. The results of the in vitro and in vivo studies show that regardless of the source, the additives improve the bioavailability of the minerals studied, so it

is proposed that natural products can help improve the nutritional characteristics of gluten-free products.

Another application of NFAs is the development of meat, fish, and derived products with the objective of improving the properties of texture, flavor or color or increasing shelf life. Different research studies have been carried out on the inclusion of different compounds, including antioxidant molecules that reduce oxidative rancidity in the product and provide it with added value [181, 182]. Ben Slima et al. [183] evaluated the inclusion of a polysaccharide with antioxidant properties in the stability of an emulsion for sausage production, finding that lipid oxidation and loss of color was lower in the samples with the natural additive, possibly because it inhibited the formation of free radicals or interrupted their propagation chain. These results suggest that natural additives can be used in the meat industry to improve the organoleptic properties of products. Rohfritsch et al. [184] evaluated the use of wheat and rice bran powders and extracts to protect the oxidation of polyunsaturated fatty acids (PUFAs) such as linoleic acid, eicosapentaenoic acid and docosahexaenoic acid present in fish oil. In an accelerated oxidation test at 38 °C, the researchers found that wheat powders showed oil protection capacity, proposing that this type of product has potential for use in the industry.

6.5 Conclusions

The production of additives from plant and microbial sources have become attractive methods for the food industry due to the fact that complex natural products can be obtained on a large scale from inexpensive raw materials. It is expected that, with the development of these technologies, the genetic modification of important microbial strains and plant varieties will increase, in addition to the discovery of new sources of microbial or plant additives, which are safe for consumers, allowing the generation of high-value products to aggregate. It is also important to note that technologies of plant and microbial origin are clean and ecological compared to those based on obtaining from chemical synthesis, which will increase their use by the food industry and related areas.

For the aforementioned reasons, it is expected that in the future, research will focus on the implementation of technologies that allow improving the production and productivity of these food additives, in addition to reducing costs. Future perspectives also point to the incorporation by the food industry of food additives obtained from microbial or vegetable origin in the elaboration of smart packaging, development of functional foods, improvement of the nutritional composition and organoleptic characteristics of the foods produced.

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Conflicts of Interest The author declares no conflict of interest.

References

1. Administration USF and D. Overview of food ingredients, additives & colors. Retrieved March. 2013;21:2013.
2. Sun L, Xin F, Alper HS. Bio-synthesis of food additives and colorants-a growing trend in future food. Biotechnol Adv. 2021;47:107694.
3. Li Z, Fan Y, Xi J. Recent advances in high voltage electric discharge extraction of bioactive ingredients from plant materials. Food Chem. 2019;277:246–60.
4. Rajha HN, Boussetta N, Louka N, Maroun RG, Vorobiev E. Effect of alternative physical pretreatments (pulsed electric field, high voltage electrical discharges and ultrasound) on the dead-end ultrafiltration of vine-shoot extracts. Sep Purif Technol. 2015;146:243–51.
5. Saguy IS, Roos YH, Cohen E. Food engineering and food science and technology: forward-looking journey to future new horizons. Innov Food Sci Emerg Technol. 2018;47:326–34.
6. Gahruie HH, Parastouei K, Mokhtarian M, Rostami H, Niakousari M, Mohsenpour Z. Application of innovative processing methods for the extraction of bioactive compounds from saffron (Crocus sativus) petals. J Appl Res Med Aromat Plants. 2020;19:100264.
7. Kunal Ahuja SS. Food additives market report. ManufacturingTomorrow. Library Catalog: www.manufacturingtomorrow.com. 2017.
8. Williams PA, Phillips GO. Introduction to food hydrocolloids. In: Handbook of hydrocolloids. Elsevier United States of America; 2021. p. 3–26.
9. Semenova M, Antipova A, Martirosova E, Zelikina D, Palmina N, Chebotarev S. Essential contributions of food hydrocolloids and phospholipid liposomes to the formation of carriers for controlled delivery of biologically active substances via the gastrointestinal tract. In: Food hydrocolloids, Vol. 120. Elsevier United States of America; 2021.
10. Li JM, Nie SP. The functional and nutritional aspects of hydrocolloids in foods. Food Hydrocoll. 2016;53:46–61.
11. Zhang N, Zhou Q, Fan D, Xiao J, Zhao Y, Cheng KW, et al. Novel roles of hydrocolloids in foods: inhibition of toxic maillard reaction products formation and attenuation of their harmful effects. Trends Food Sci Technol Elsevier Ltd. 2021;111:706–15.
12. Manzoor M, Singh J, Bandral JD, Gani A, Shams R. Food hydrocolloids: functional, nutraceutical and novel applications for delivery of bioactive compounds. Int J Biol Macromol Elsevier BV. 2020;165:554–67.
13. Pereira L. Macroalgae. Encyclopedia. 2021;1(1):177–88.
14. Rhein-Knudsen N, Meyer AS. Chemistry, gelation, and enzymatic modification of seaweed food hydrocolloids. In: Trends in food science and technology, Vol. 109. Elsevier United States of America; 2021. p. 608–621.
15. Mahmood K, Kamilah H, Shang PL, Sulaiman S, Ariffin F. A review: interaction of starch/non-starch hydrocolloid blending and the recent food applications. Food Biosci. 2017;19:110–20.
16. Krstonošić V, Jovičić-Bata J, Maravić N, Nikolić I, Dokić L. Rheology, structure, and sensory perception of hydrocolloids. In: Food structure and functionality. Elsevier United States of America; 2021. p. 23–47.
17. Ifuku S, Nomura R, Morimoto M, Saimoto H. Preparation of chitin nanofibers from mushrooms. Materials (Basel). 2011;4(8):1417–25.
18. Ghormade V, Pathan EK, Deshpande MV. Can fungi compete with marine sources for chitosan production? Int J Biol Macromol. 2017;104:1415–21.
19. Hassainia A, Satha H, Boufi S. Chitin from Agaricus bisporus: extraction and characterization. Int J Biol Macromol. 2018;117:1334–42.
20. Di Mario F, Rapana P, Tomati U, Galli E. Chitin and chitosan from Basidiomycetes. Int J Biol Macromol. 2008;43(1):8–12.
21. Yasrebi N, Hatamian Zarmi AS, Larypoor M. Optimization of chitosan production from Iranian medicinal fungus trametes-versicolor by Taguchi method and evaluation of antibacterial properties. Iran J Med Microbiol. 2020;14(3):186–200.

22. Johney J, Eagappan K, Ragunathan RR. Microbial extraction of chitin and chitosan from Pleurotus spp, its characterization and antimicrobial activity. Int J Curr Pharm Res. 2016;9(1):88–93.
23. Zielinski AAF, del Pilar S-CA, Benvenutti L, Ferro DM, Dias JL, Ferreira SRS. High-pressure fluid technologies: recent approaches to the production of natural pigments for food and pharmaceutical applications. Trends Food Sci Technol. 2021;118:850–69.
24. Benavente-Valdés JR, Méndez-Zavala A, Hernández-López I, Carreón-González BA, Velázquez-Arellano ME, Morales-Oyervides L, et al. Unconventional microalgae species and potential for their use in the food industry. In: Cultured microalgae for the food industry. Elsevier United States of America; 2021. p. 49–71.
25. Nuhu AA. Spirulina (Arthrospira): an important source of nutritional and medicinal compounds. J Mar Biol. 2013;2013:1–8.
26. Vali Aftari R, Rezaei K, Mortazavi A, Bandani AR. The optimized concentration and purity of Spirulina platensis C-phycocyanin: a comparative study on microwave-assisted and ultrasound-assisted extraction methods. J Food Process Preserv. 2015;39(6):3080–91.
27. Glazer AN. Phycobiliproteins—a family of valuable, widely used fluorophores. J Appl Phycol. 1994;6(2):105–12.
28. Pan-utai W, Iamtham S. Extraction, purification and antioxidant activity of phycobiliprotein from Arthrospira platensis. Process Biochem. 2019;82:189–98.
29. Prasanna R, Kumar R, Sood A, Prasanna BM, Singh PK. Morphological, physiochemical and molecular characterization of Anabaena strains. Microbiol Res. 2006;161(3):187–202.
30. Acquah C, Ekezie F-G, Udenigwe CC. Potential applications of microalgae-derived proteins and peptides in the food industry. In: Cultured microalgae for the food industry. Elsevier United States of America; 2021. p. 97–126.
31. Aruldass CA, Dufossé L, Ahmad WA. Current perspective of yellowish-orange pigments from microorganisms-a review. J Clean Prod. 2018;180:168–82.
32. Dos Santos MC, Bicas JL. Natural blue pigments and bikaverin. Microbiol Res. 2021;244:126653.
33. Carlsen MH, Halvorsen BL, Holte K, Bøhn SK, Dragland S, Sampson L, et al. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutr J. 2010;9(1):1–11.
34. Vieira MV, Oliveira SM, Amado IR, Fasolin LH, Vicente AA, Pastrana LM, et al. 3D printed functional cookies fortified with Arthrospira platensis: evaluation of its antioxidant potential and physical-chemical characterization. Food Hydrocoll. 2020;107:105893.
35. Kharkongor D, Ramanujam P. Antioxidant activities of four dominant species of Trentepohlia (Trentepohliales, Chlorophyta). Int J Complement Altern Med. 2017;8(5):270.
36. Matos ÂP. Microalgae as a potential source of proteins. In: Proteins: sustainable source, processing and applications. Elsevier United States of America; 2019. p. 63–96.
37. Arora DS, Sharma RK, Chandra P. Biodelignification of wheat straw and its effect on in vitro digestibility and antioxidant properties. Int Biodeterior Biodegradation. 2011;65(2):352–8.
38. Janardhan A, Kumar AP, Viswanath B, Saigopal DVR, Narasimha G. Production of bioactive compounds by actinomycetes and their antioxidant properties. Biotechnol Res Int. 2014;2014:1–8.
39. Angelin J, Kavitha M. Exopolysaccharides from probiotic bacteria and their health potential. Int J Biol Macromol. 2020;162:853–65.
40. Chandra P, Sharma RK, Arora DS. Antioxidant compounds from microbial sources: a review. Food Res Int. 2020;129:108849.
41. Anwar H, Hussain G, Mustafa I. Antioxidants from natural sources. Antioxidants Foods Appl. 2018;1:3–28.
42. Fleming E, Luo Y. Co-delivery of synergistic antioxidants from food sources for the prevention of oxidative stress. J Agric Food Res. 2021;3:100107.

43. Sir Elkhatim KA, Elagib RAA, Hassan AB. Content of phenolic compounds and vitamin C and antioxidant activity in wasted parts of Sudanese citrus fruits. Food Sci Nutr. 2018;6(5):1214–9.
44. Dimou C, Karantonis HC, Skalkos D, Koutelidakis AE. Valorization of fruits by-products to unconventional sources of additives, oil, biomolecules and innovative functional foods. Curr Pharm Biotechnol. 2019;20(10):776–86.
45. Bobinaitė R, Viskelis P, Bobinas Č, Mieželienė A, Alenčikienė G, Venskutonis PR. Raspberry marc extracts increase antioxidative potential, ellagic acid, ellagitannin and anthocyanin concentrations in fruit purees. LWT-Food Sci Technol. 2016;66:460–7.
46. Kim W, Wang Y, Selomulya C. Dairy and plant proteins as natural food emulsifiers. Trends Food Sci Technol. 2020;105:261–72.
47. Robert C, Couëdelo L, Vaysse C, Michalski M-C. Vegetable lecithins: a review of their compositional diversity, impact on lipid metabolism and potential in cardiometabolic disease prevention. Biochimie. 2020;169:121–32.
48. Miller R. Emulsifiers: types and uses. In: Caballero B, Finglas PM, Toldra F, editors. Encyclopedia of food and health. Elsevier United States of America; 2016.
49. Wang M, Yan W, Zhou Y, Fan L, Liu Y, Li J. Progress in the application of lecithins in water-in-oil emulsions. Trends Food Sci Technol. 2021;118:388–98.
50. Burger TG, Zhang Y. Recent progress in the utilization of pea protein as an emulsifier for food applications. Trends Food Sci Technol. 2019;86:25–33.
51. McClements DJ, Grossmann L. A brief review of the science behind the design of healthy and sustainable plant-based foods. NPJ Sci food. 2021;5(1):1–10.
52. Pessôa MG, Vespermann KAC, Paulino BN, Barcelos MCS, Pastore GM, Molina G. Newly isolated microorganisms with potential application in biotechnology. Biotechnol Adv. 2019;37(2):319–39.
53. Zouari R, Besbes S, Ellouze-Chaabouni S, Ghribi-Aydi D. Cookies from composite wheat–sesame peels flours: dough quality and effect of Bacillus subtilis SPB1 biosurfactant addition. Food Chem. 2016;194:758–69.
54. Campos JM, Stamford TLM, Rufino RD, Luna JM, Stamford TCM, Sarubbo LA. Formulation of mayonnaise with the addition of a bioemulsifier isolated from Candida utilis. Toxicol Rep. 2015;2:1164–70.
55. Nitschke M, Silva SSE. Recent food applications of microbial surfactants. Crit Rev Food Sci Nutr. 2018;58(4):631–8.
56. Alizadeh-Sani M, Hamishehkar H, Khezerlou A, Azizi-Lalabadi M, Azadi Y, Nattagh-Eshtivani E, et al. Bioemulsifiers derived from microorganisms: applications in the drug and food industry. Adv Pharm Bull. 2018;8(2):191.
57. Aronson JKBT-MSE of D. Vitamin E. In: Sixteenth E, editor. . Oxford: Elsevier; 2016. p. 488–93. Available from: https://www.sciencedirect.com/science/article/pii/B9780444537171016401.
58. Durmaz Y, Vitamin E. (α-tocopherol) production by the marine microalgae Nannochloropsis oculata (Eustigmatophyceae) in nitrogen limitation. Aquaculture. 2007;272(1–4):717–22.
59. Revuelta JL, Buey RM, Ledesma-Amaro R, Vandamme EJ. Microbial biotechnology for the synthesis of (pro) vitamins, biopigments and antioxidants: challenges and opportunities. Microb Biotechnol. 2016;9(5):564–7.
60. Stahmann K-P. Vitamins and vitamin-like compounds: microbial production. 2019.
61. Wu X, Zha J, Koffas MAG. Microbial production of bioactive chemicals for human health. Curr Opin Food Sci. 2020;32:9–16.
62. Averianova LA, Balabanova LA, Son OM, Podvolotskaya AB, Tekutyeva LA. Production of vitamin B2 (riboflavin) by microorganisms: an overview. Front Bioeng Biotechnol. 2020;1172:1–23.
63. Bellou S, Triantaphyllidou I-E, Aggeli D, Elazzazy AM, Baeshen MN, Aggelis G. Microbial oils as food additives: recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. Curr Opin Biotechnol. 2016;37:24–35.

64. Katiyar R, Arora A. Health promoting functional lipids from microalgae pool: a review. Algal Res. 2020;46:101800.
65. Gong Y, Wan X, Jiang M, Hu C, Hu H, Huang F. Metabolic engineering of microorganisms to produce omega-3 very long-chain polyunsaturated fatty acids. Prog Lipid Res. 2014;56:19–35.
66. Ruiz-Lopez N, Haslam RP, Usher SL, Napier JA, Sayanova O. Reconstitution of EPA and DHA biosynthesis in Arabidopsis: iterative metabolic engineering for the synthesis of n− 3 LC-PUFAs in transgenic plants. Metab Eng. 2013;17:30–41.
67. Ruiz-Lopez N, Haslam RP, Napier JA, Sayanova O. Successful high-level accumulation of fish oil omega-3 long-chain polyunsaturated fatty acids in a transgenic oilseed crop. Plant J. 2014;77(2):198–208.
68. Morabito C, Bournaud C, Maës C, Schuler M, Cigliano RA, Dellero Y, et al. The lipid metabolism in thraustochytrids. Prog Lipid Res. 2019;76:101007.
69. Meesapyodsuk D, Qiu X. Biosynthetic mechanism of very long chain polyunsaturated fatty acids in Thraustochytrium sp. 26185 [S]. J Lipid Res. 2016;57(10):1854–64.
70. Joffre C, Viau S. Dietary N-3 polyunsaturated fatty acids and dry eye. In: Handbook of nutrition, diet and the eye. Elsevier United States of America; 2014. p. 177–187.
71. Diao J, Song X, Guo T, Wang F, Chen L, Zhang W. Cellular engineering strategies toward sustainable omega-3 long chain polyunsaturated fatty acids production: state of the art and perspectives. Biotechnol Adv. 2020;40:107497.
72. Ozogul F, Ozcelik S, Ozogul Y, Yilmaz MT. Seafood infusion broths as novel sources to produce organic acids using selected lactic acid bacteria strains. Food Biosci. 2021;43:101227.
73. Kallscheuer N. Engineered microorganisms for the production of food additives approved by the European Union—a systematic analysis. Front Microbiol. 2018;9:1746.
74. Panda SK, Mishra SS, Kayitesi E, Ray RC. Microbial-processing of fruit and vegetable wastes for production of vital enzymes and organic acids: biotechnology and scopes. Environ Res. 2016;146:161–72.
75. Zelle RM, De Hulster E, Van Winden WA, De Waard P, Dijkema C, Winkler AA, et al. Malic acid production by Saccharomyces cerevisiae: engineering of pyruvate carboxylation, oxaloacetate reduction, and malate export. Appl Environ Microbiol. 2008;74(9):2766–77.
76. West TP. Microbial production of malic acid from biofuel-related coproducts and biomass. Fermentation. 2017;3(2):14.
77. Engel CAR, Van Gulik WM, Marang L, Van der Wielen LAM, Straathof AJJ. Development of a low pH fermentation strategy for fumaric acid production by Rhizopus oryzae. Enzym Microb Technol. 2011;48(1):39–47.
78. Oren A. Microbial metabolism: importance for environmental biotechnology. In: Environmental biotechnology. Springer United States of America; 2010. p. 193–255.
79. Ladakis D, Papapostolou H, Vlysidis A, Koutinas A. Inventory of food processing side streams in European Union and prospects for biorefinery development. In: Food industry wastes. Elsevier United States of America; 2020. p. 181–199.
80. Thakker C, Martínez I, San K, Bennett GN. Succinate production in Escherichia coli. Biotechnol J. 2012;7(2):213–24.
81. Ali S, Qadeer MA, Iqbal J. Citric acid fermentation by mutant strain of Aspergillus niger GCMC-7using molasses based medium. Electron J Biotechnol. 2002;5(2):8–9.
82. Karaffa L, Kubicek CP. Aspergillus niger citric acid accumulation: do we understand this well working black box? Appl Microbiol Biotechnol. 2003;61(3):189–96.
83. de Carvalho CCCR, da Fonseca MMR. 2.33@_ Biotransformations. In 2011.
84. Ben AN, Gargouri M. Microbial and enzymatic technologies used for the production of natural aroma compounds: synthesis, recovery modeling, and bioprocesses. Food Bioprod Process. 2015;94:675–706.
85. Christen P, Meza JC, Revah S. Fruity aroma production in solid state fermentation by Ceratocystis fimbriata: influence of the substrate type and the presence of precursors. Mycol Res. 1997;101(8):911–9.

86. Soares M, Christen P, Pandey A, Soccol CR. Fruity flavour production by Ceratocystis fimbriata grown on coffee husk in solid-state fermentation. Process Biochem. 2000;35(8):857–61.
87. Maria I, Elena B, Alexandru S, Corina PE. Using microbial systems in order to obtain fermentation flavorings. J Agroal Proc Technol. 2009;15(1):34–40.
88. Braga A, Guerreiro C, Belo I. Generation of flavors and fragrances through biotransformation and de novo synthesis. Food Bioprocess Technol. 2018;11(12):2217–28.
89. Raveendran S, Parameswaran B, Ummalyma SB, Abraham A, Mathew AK, Madhavan A, et al. Applications of microbial enzymes in food industry. Food Technol Biotechnol. 2018;56(1):16.
90. Van Der Maarel MJEC, Van der Veen B, Uitdehaag JCM, Leemhuis H, Dijkhuizen L. Properties and applications of starch-converting enzymes of the α-amylase family. J Biotechnol. 2002;94(2):137–55.
91. Coutinho PM, Reilly PJ. Glucoamylase structural, functional, and evolutionary relationships. Prot Struct Funct Bioinform. 1997;29(3):334–47.
92. Deng L, Wang Z, Yang S, Song J, Que F, Zhang H, et al. Improvement of functional properties of wheat gluten using acid protease from Aspergillus usamii. PLoS One. 2016;11(7):e0160101.
93. Jurado E, Camacho F, Luzon G, Vicaria JM. A new kinetic model proposed for enzymatic hydrolysis of lactose by a β-galactosidase from Kluyveromyces fragilis. Enzym Microb Technol. 2002;31(3):300–9.
94. Krishania M, Sindhu R, Binod P, Ahluwalia V, Kumar V, Sangwan RS, et al. Design of bioreactors in solid-state fermentation. In: Current developments in biotechnology and bioengineering. Elsevier United States of America; 2018. p. 83–96.
95. De Maria L, Vind J, Oxenbøll KM, Svendsen A, Patkar S. Phospholipases and their industrial applications. Appl Microbiol Biotechnol. 2007;74(2):290–300.
96. Alvarez-Macarie E, Baratti J. Short chain flavour ester synthesis by a new esterase from Bacillus licheniformis. J Mol Catal B Enzym. 2000;10(4):377–83.
97. Patel DD, Patel RR, Thakkar VR. Purification, characterization and application of lipoxygenase isoenzymes from Lasiodiplodia theobromae. Appl Biochem Biotechnol. 2015;175(1):513–25.
98. Mandal A. Review on microbial xylanases and their applications. Appl Microbiol Biotechnol. 2015;42:42–5.
99. Kona RP, Qureshi N, Pai JS. Production of glucose oxidase using Aspergillus niger and corn steep liquor. Bioresour Technol. 2001;78(2):123–6.
100. Morozova OV, Shumakovich GP, Shleev SV, Yaropolov YI. Laccase-mediator systems and their applications: a review. Appl Biochem Microbiol. 2007;43(5):523–35.
101. Sadhasivam S, Savitha S, Swaminathan K, Lin F-H. Production, purification and characterization of mid-redox potential laccase from a newly isolated Trichoderma harzianum WL1. Process Biochem. 2008;43(7):736–42.
102. Puri M, Sharma D, Barrow CJ. Enzyme-assisted extraction of bioactives from plants. Trends Biotechnol. 2012;30(1):37–44.
103. Jayant D, Halami PM. Industrial perspective of food preservatives from microbial origin. In: Current developments in biotechnology and bioengineering. Elsevier United States of America; 2020. p. 243–261.
104. Khorshidian N, Yousefi M, Khanniri E, Mortazavian AM. Potential application of essential oils as antimicrobial preservatives in cheese. Innov Food Sci Emerg Technol. 2018;45:62–72.
105. Falleh H, Ben JM, Saada M, Ksouri R. Essential oils: a promising eco-friendly food preservative. Food Chem. 2020;330:127268.
106. Friedman M. Chemistry and multibeneficial bioactivities of carvacrol (4-isopropyl-2-methylphenol), a component of essential oils produced by aromatic plants and spices. J Agric Food Chem. American Chemical Society. 2014;62:7652–70.
107. Saeed F, Afzaal M, Tufail T, Ahmad A. Use of natural antimicrobial agents: a safe preservation approach. Act Antimicrob Food Packag. 2019;18:1–104.

108. Singh VP. Recent approaches in food bio-preservation-a review. Open Vet J. 2018;8(1):104–11.
109. Zhang Y, Chen H, Pan K. Chapter 5—Nanoencapsulation of food antimicrobial agents and essential oils. In: Jafari SM, editor. Nanoencapsulation food bioact ingredients. Cambridge, MA: Academic Press; 2017. p. 183–221.
110. Amanullah A, Satti S, Nienow AW. Enhancing xanthan fermentations by different modes of glucose feeding. Biotechnol Prog. 1998;14(2):265–9.
111. Zhu G, Sheng L, Tong Q. Enhanced gellan gum production by hydrogen peroxide (H2O2) induced oxidative stresses in Sphingomonas paucimobilis. Bioprocess Biosyst Eng. 2014;37(4):743–8.
112. Kamer DDA, Gumus T, Palabiyik I, Demirci AS, Oksuz O. Grape pomace as a promising source for gellan gum production. Food Hydrocoll. 2021;114:106584.
113. Martínez-Sanz M, Gómez-Mascaraque LG, Ballester AR, Martínez-Abad A, Brodkorb A, López-Rubio A. Production of unpurified agar-based extracts from red seaweed Gelidium sesquipedale by means of simplified extraction protocols. Algal Res. 2019;38:101420.
114. Li Y, Zhao M, Gomez LP, Senthamaraikannan R, Padamati RB, O'Donnell CP, et al. Investigation of enzyme-assisted methods combined with ultrasonication under a controlled alkali pretreatment for agar extraction from Gelidium sesquipedale. Food Hydrocoll. 2021;120:106905.
115. Crugeira PJL, de Almeida PF, Sampaio ICF, Soares LGP, Amador DAM, Samuel IDW, et al. Production and viscosity of Xanthan Gum are increased by LED irradiation of X. campestris cultivated in medium containing produced water of the oil industry. J Photochem Photobiol B Biol. 2022;226:112356.
116. Wang D, Kim H, Lee S, Kim D-H, Joe M-H. Improved gellan gum production by a newly-isolated Sphingomonas azotifigens GL-1 in a cheese whey and molasses based medium. Process Biochem. 2020;95:269–78.
117. Valladares-Diestra KK, de Souza Vandenberghe LP, Torres LAZ, Zandoná Filho A, Woiciechowski AL, Soccol CR. Citric acid assisted hydrothermal pretreatment for the extraction of pectin and xylooligosaccharides production from cocoa pod husks. Bioresour Technol. 2022;343:126074.
118. de Oliveira CF, Giordani D, Gurak PD, Cladera-Olivera F, Marczak LDF. Extraction of pectin from passion fruit peel using moderate electric field and conventional heating extraction methods. Innov Food Sci Emerg Technol. 2015;29:201–8.
119. Karanicola P, Patsalou M, Stergiou P-Y, Kavallieratou A, Evripidou N, Christou P, et al. Ultrasound-assisted dilute acid hydrolysis for production of essential oils, pectin and bacterial cellulose via a citrus processing waste biorefinery. Bioresour Technol. 2021;342:126010.
120. Bao Y, Reddivari L, Huang J-Y. Development of cold plasma pretreatment for improving phenolics extractability from tomato pomace. Innov Food Sci Emerg Technol. 2020;65:102445.
121. Banjo T, Kareem S, Akinduti P, Popoola T, Akinloye O. Optimization and production of ascorbic acid by fusant cell of Aspergillus flavus and Aspergillus tamarii. J King Saud Univ. 2019;31(4):931–6.
122. Reshma R, Devi KC, Kumar SD, Santhanam P, Perumal P, Krishnaveni N, et al. Enhancement of pigments production in the green microalga Dunaliella salina (PSBDU05) under optimized culture condition. Bioresour Technol Rep. 2021;14:100672.
123. Shetty AVK, Dave N, Murugesan G, Pai S, Pugazhendhi A, Varadavenkatesan T, et al. Production and extraction of red pigment by solid-state fermentation of broken rice using Monascus sanguineus NFCCI 2453. Biocatal Agric Biotechnol. 2021;33:101964.
124. Chen X, Yan J, Chen J, Gui R, Wu Y, Li N. Potato pomace: an efficient resource for Monascus pigments production through solid-state fermentation. J Biosci Bioeng. 2021;132(2):167–73.
125. Manikprabhu D, Lingappa K. γ Actinorhodin a natural and attorney source for synthetic dye to detect acid production of fungi. Saudi J Biol Sci. 2013;20(2):163–8.

126. Fialho AM, Bernardes N, Chakrabarty AM. Exploring the anticancer potential of the bacterial protein azurin. AIMS Microbiol. 2016;2(3):292–303.
127. Yusuf M. Agro-industrial waste materials and their recycled value-added applications. In: Handb Ecomater; 2017. p. 1.
128. Cude WN, Mooney J, Tavanaei AA, Hadden MK, Frank AM, Gulvik CA, et al. Production of the antimicrobial secondary metabolite indigoidine contributes to competitive surface colonization by the marine roseobacter Phaeobacter sp. strain Y4I. Appl Environ Microbiol. 2012;78(14):4771–80.
129. Koch B, Steglich W. Meroterpenoid pigments from Albatrellus flettii (Basidiomycetes). Wiley Online Library; 2007.
130. Ferreira GF, Pessoa JGB, Pinto LFR, Maciel Filho R, Fregolente LV. Mono-and diglyceride production from microalgae: challenges and prospects of high-value emulsifiers. Trends Food Sci Technol. 2021;118:589–600.
131. Tarento TDC, McClure DD, Vasiljevski E, Schindeler A, Dehghani F, Kavanagh JM. Microalgae as a source of vitamin K1. Algal Res. 2018;36:77–87.
132. Volker AR, Gogerty DS, Bartholomay C, Hennen-Bierwagen T, Zhu H, Bobik TA. Fermentative production of short-chain fatty acids in Escherichia coli. Microbiology. 2014;160(7):1513–22.
133. Coban HB, Demirci A. Enhancement and modeling of microparticle-added Rhizopus oryzae lactic acid production. Bioprocess Biosyst Eng. 2016;39(2):323–30.
134. Zhang Y, Abatzoglou N. Fundamentals, applications and potentials of ultrasound-assisted drying. Chem Eng Res Des. 2020;154:21–46.
135. Pimentel-Moral S, Borrás-Linares I, Lozano-Sánchez J, Arráez-Román D, Martínez-Férez A, Segura-Carretero A. Microwave-assisted extraction for Hibiscus sabdariffa bioactive compounds. J Pharm Biomed Anal. 2018;156:313–22.
136. Ma Y, Wu X, Zhao L, Wang Y, Liao X. Comparison of the compounds and characteristics of pepper seed oil by pressure-assisted, ultrasound-assisted and conventional solvent extraction. Innov Food Sci Emerg Technol. 2019;54:78–86.
137. Martínez-Ramos T, Benedito-Fort J, Watson NJ, Ruiz-López II, Che-Galicia G, Corona-Jiménez E. Effect of solvent composition and its interaction with ultrasonic energy on the ultrasound-assisted extraction of phenolic compounds from Mango peels (Mangifera indica L.). Food Bioprod Process. 2020;122:41–54.
138. Tutunchi P, Roufegarinejad L, Hamishehkar H, Alizadeh A. Extraction of red beet extract with β-cyclodextrin-enhanced ultrasound assisted extraction: a strategy for enhancing the extraction efficacy of bioactive compounds and their stability in food models. Food Chem. 2019;297:124994.
139. Wang F, Zhang Y, Xu L, Ma H. An efficient ultrasound-assisted extraction method of pea protein and its effect on protein functional properties and biological activities. Lwt. 2020;127:109348.
140. Meregalli MM, Puton BMS, Camera FD, Amaral AU, Zeni J, Cansian RL, et al. Conventional and ultrasound-assisted methods for extraction of bioactive compounds from red araçá peel (Psidium cattleianum Sabine). Arab J Chem. 2020;13(6):5800–9.
141. Albuquerque KKSA, Albuquerque WWC, Costa RMPB, Batista JMS, Marques DA V, Bezerra RP, et al. Biotechnological potential of a novel tannase-acyl hydrolase from Aspergillus sydowii using waste coir residue: aqueous two-phase system and chromatographic techniques. Biocatal Agric Biotechnol. 2020;23:101453.
142. Patil SS, Pathak A, Rathod VK. Optimization and kinetic study of ultrasound assisted deep eutectic solvent based extraction: a greener route for extraction of curcuminoids from Curcuma longa. Ultrason Sonochem. 2021;70:105267.
143. Karğılı U, Aytaç E. Supercritical fluid extraction of cannabinoids (THC and CBD) from four different strains of cannabis grown in different regions. J Supercrit Fluids. 2022;179:105410.
144. Picot-Allain C, Mahomoodally MF, Ak G, Zengin G. Conventional versus green extraction techniques—a comparative perspective. Curr Opin Food Sci. 2021;40:144–56.

145. Rodríguez-España M, Mendoza-Sánchez LG, Magallón-Servín P, Salgado-Cervantes MA, Acosta-Osorio AA, García HS. Supercritical fluid extraction of lipids rich in DHA from Schizochytrium sp. J Supercrit Fluids. 2022;179:105391.
146. Gong T, Liu S, Wang H, Zhang M. Supercritical CO2 fluid extraction, physicochemical properties, antioxidant activities and hypoglycemic activity of polysaccharides derived from fallen Ginkgo leaves. Food Biosci. 2021;42:101153.
147. do Espirito Santo AT, Siqueira LM, Almeida RN, RMF V, do Franceschini NG, Kunde MA, et al. Decaffeination of yerba mate by supercritical fluid extraction: improvement, mathematical modelling and infusion analysis. J Supercrit Fluids. 2021;168:105096.
148. Mwaurah PW, Kumar S, Kumar N, Attkan AK, Panghal A, Singh VK, et al. Novel oil extraction technologies: process conditions, quality parameters, and optimization. Compr Rev Food Sci Food Saf. 2020;19(1):3–20.
149. Hu B, Xi X, Li H, Qin Y, Li C, Zhang Z, et al. A comparison of extraction yield, quality and thermal properties from Sapindus mukorossi seed oil between microwave assisted extraction and Soxhlet extraction. Ind Crop Prod. 2021;161:113185.
150. Liu Z, Li H, Qi Y, Zhu Z, Huang D, Zhang K, et al. Cinnamomum camphora leaves as a source of proanthocyanidins separated using microwave-assisted extraction method and evaluation of their antioxidant activity in vitro. Arab J Chem. 2021;14(9):103328.
151. Figueroa JG, Borrás-Linares I, Del Pino-García R, Curiel JA, Lozano-Sánchez J, Segura-Carretero A. Functional ingredient from avocado peel: microwave-assisted extraction, characterization and potential applications for the food industry. Food Chem. 2021;352:129300.
152. Domínguez-Rodríguez G, Marina ML, Plaza M. Enzyme-assisted extraction of bioactive non-extractable polyphenols from sweet cherry (Prunus avium L.) pomace. Food Chem. 2021;339:128086.
153. de Queirós LD, Dias FFG, de Ávila ARA, Macedo JA, Macedo GA, de Moura JMLN. Effects of enzyme-assisted extraction on the profile and bioaccessibility of isoflavones from soybean flour. Food Res Int. 2021;147:110474.
154. Song Y-R, Han A-R, Park S-G, Cho C-W, Rhee Y-K, Hong H-D. Effect of enzyme-assisted extraction on the physicochemical properties and bioactive potential of lotus leaf polysaccharides. Int J Biol Macromol. 2020;153:169–79.
155. Rezaei S, Ebadi M-T, Ghobadian B, Ghomi H. Optimization of DBD-Plasma assisted hydrodistillation for essential oil extraction of fennel (Foeniculum vulgare Mill.) seed and spearmint (Mentha spicata L.) leaf. J Appl Res Med Aromat Plants. 2021;24:100300.
156. da Loureiro AC, das do Souza FCA, Sanches EA, de Bezerra JA, Lamarão CV, Rodrigues S, et al. Cold plasma technique as a pretreatment for drying fruits: evaluation of the excitation frequency on drying process and bioactive compounds. Food Res Int. 2021;147:110462.
157. Käferböck A, Smetana S, de Vos R, Schwarz C, Toepfl S, Parniakov O. Sustainable extraction of valuable components from Spirulina assisted by pulsed electric fields technology. Algal Res. 2020;48:101914.
158. Siloto RMP, Weselake RJ. Site saturation mutagenesis: methods and applications in protein engineering. Biocatal Agric Biotechnol. 2012;1(3):181–9.
159. Visockis M, Bobinaitė R, Ruzgys P, Barakauskas J, Markevičius V, Viškelis P, et al. Assessment of plant tissue disintegration degree and its related implications in the pulsed electric field (PEF)–assisted aqueous extraction of betalains from the fresh red beetroot. Innov Food Sci Emerg Technol. 2021;73:102761.
160. Niu D, Ren E-F, Li J, Zeng X-A, Li S-L. Effects of pulsed electric field-assisted treatment on the extraction, antioxidant activity and structure of naringin. Sep Purif Technol. 2021;265:118480.
161. de Oliveira CF, Giordani D, Lutckemier R, Gurak PD, Cladera-Olivera F, Marczak LDF. Extraction of pectin from passion fruit peel assisted by ultrasound. LWT-Food Sci Technol. 2016;71:110–5.

162. Priya NV, Vinitha UG, Sundaram MM. Preparation of chitosan-based antimicrobial active food packaging film incorporated with Plectranthus amboinicus essential oil. Biocatal Agric Biotechnol. 2021;34:102021.
163. Silva-Weiss A, Ihl M, Sobral PJ, do Gómez-Guillén AMC, Bifani V. Natural additives in bioactive edible films and coatings: functionality and applications in foods. Food Eng Rev. 2013;5(4):200–16.
164. Moraczewski K, Pawłowska A, Stepczyńska M, Malinowski R, Kaczor D, Budner B, et al. Plant extracts as natural additives for environmentally friendly polylactide films. Food Packag Shelf Life. 2020;26:100593.
165. de Oliveira F, de Lima CA, Lopes AM, de Marques DAV, Druzian JI, Pessoa Júnior A, et al. Microbial colorants production in stirred-tank bioreactor and their incorporation in an alternative food packaging biomaterial. J Fungi. 2020;6(4):264.
166. Moradi M, Guimarães JT, Sahin S. Current applications of exopolysaccharides from lactic acid bacteria in the development of food active edible packaging. Curr Opin Food Sci. 2021;40:33–9.
167. Moradi M, Kousheh SA, Almasi H, Alizadeh A, Guimarães JT, Yılmaz N, et al. Postbiotics produced by lactic acid bacteria: the next frontier in food safety. Compr Rev Food Sci Food Saf. 2020;19(6):3390–415.
168. İncili GK, Karatepe P, Akgöl M, Kaya B, Kanmaz H, Hayaloğlu AA. Characterization of Pediococcus acidilactici postbiotic and impact of postbiotic-fortified chitosan coating on the microbial and chemical quality of chicken breast fillets. Int J Biol Macromol. 2021;184:429–37.
169. Alongi M, Anese M. Re-thinking functional food development through a holistic approach. J Funct Foods. 2021;81:104466.
170. Yessuf AM, Srinivasan B, Eranna BC. Development of functional drink from the leaves of Moringa Stenopetala. J Chem Chem Sci. 2020;10(8):296–310.
171. Corsetto PA, Montorfano G, Zava S, Colombo I, Ingadottir B, Jonsdottir R, et al. Characterization of antioxidant potential of seaweed extracts for enrichment of convenience food. Antioxidants. 2020;9(3):249.
172. Poshadri A, Aparna K. Microencapsulation technology: a review. J Res ANGRAU. 2010;38(1):86–102.
173. Delshadi R, Bahrami A, Tafti AG, Barba FJ, Williams LL. Micro and nano-encapsulation of vegetable and essential oils to develop functional food products with improved nutritional profiles. Trends Food Sci Technol. 2020;104:72–83.
174. Kumar LRG, Sanath Kumar H, Tejpal CS, Anas KK, Nayak BB, Sarika K, et al. Exploring the physical and quality attributes of muffins incorporated with microencapsulated squalene as a functional food additive. J Food Sci Technol. 2021;58(12):4674–84.
175. Tzima K, Brunton NP, Choudhary A, Rai DK. Potential applications of polyphenols from Herbs and spices in dairy products as natural antioxidants. In: Herbs, Spices Med Plants Process Heal Benefits Saf; 2020. p. 283–299.
176. Alibekov RS, Gabrilyants EA, Utebaeva AA, Nurseitova ZT, Konarbayeva ZK, Khamitova BM. Cottage cheese fortified by natural additives. Food Res. 2021;5(1):152–9.
177. Quintana Martínez SE, Torregroza Fuentes EE, García Zapateiro LA. Food hydrocolloids from butternut squash (Cucurbita moschata) peel: rheological properties and their use in Carica papaya jam. ACS Omega. 2021;6(18):12114–23.
178. El-Saadony MT, Elsadek MF, Mohamed AS, Taha AE, Ahmed BM, Saad AM. Effects of chemical and natural additives on cucumber juice's quality, shelf life, and safety. Foods. 2020;9(5):639.
179. Vega EN, Molina AK, Pereira C, Dias MI, Heleno SA, Rodrigues P, et al. Anthocyanins from rubus fruticosus l. And morus nigra l. applied as food colorants: a natural alternative. Plan Theory. 2021;10(6):1181.

180. Rogaska A, Reguła J, Sulibursk a J, Krejpcio Z. A comparative study of the bioavailability of Fe, Cu and Zn from Gluten-free breads enriched with natural and synthetic additives. Foods. 2020;9(12):1853.
181. Domínguez R, Gullón P, Pateiro M, Munekata PES, Zhang W, Lorenzo JM. Tomato as potential source of natural additives for meat industry. A review. Antioxidants. 2020;9(1):73.
182. Pateiro M, Gómez-Salazar JA, Jaime-Patlán M, Sosa Morales ME, Lorenzo JM. Plant extracts obtained with green solvents as natural antioxidants in fresh meat products. Antioxidants. 2021;10(2):181.
183. Ben SS, Ktari N, Trabelsi I, Moussa H, Makni I, Ben SR. Purification, characterization and antioxidant properties of a novel polysaccharide extracted from Sorghum bicolor (L.) seeds in sausage. Int J Biol Macromol. 2018;106:168–78.
184. Rohfritsch Z, Canelli G, Pollien P, Bel-Rhlid R. Wheat and rice bran as natural additives for the protection of fish oil from oxidation. ACS Food Sci Technol. 2021;1(7):1160–8.

Chapter 7
Utilization of *Saccharomyces cerevisiae* as a Source of Natural Food Additives

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7.1 Introduction

Saccharomyces cerevisiae is a unicellular fungus, possessing a completely sequenced genome with a DNA of 12068 kilobases (kb) organized in 16 chromosomes [1]. It was found to contain approximately 6000 genes, of which, 5570 are predicted to be protein encoding genes. Winemaking, baking, distilling, and brewing are the main applications of the budding yeast *S. cerevisiae* for tens of centuries [2]. The Neolithic times (about 9000 years ago) is the earliest evidence for fermented wine-like beverage production [3]. In a study of Duan et al. [2] a set of wild and fermentation associated *S. cerevisiae* isolates from diversified sources in China were analyzed. According to these authors, the results showed that China or more broadly Far East Asia is likely the origin center of *Saccharomyces* yeasts.

S. cerevisiae can be found in the wild in different environments and are often subjected to a shortage of food [4]. Oak trees (*Quercus spp*., Fagaceae family), represent a natural niche where *S. cerevisiae*, together with closely related species, has frequently been experimented across the world [5]. Besides environments of breweries and bakeries, another set of *S. cerevisiae* strains has an even more intimate association with humans, mainly colonizing the human gut environment [4].

The morphology of yeasts described by Reed and Nagodawithana, [6] demonstrates predominant shapes are oval or elliptical, with variable dimensions, presenting about 4 to 8 μm in the smallest diameter by 5 to 16 μm in the largest diameter, depending on the composition of the culture medium. The yeast has a relatively high content of minerals, varying between 9.8 and 14.4%, with phosphorus and potassium being the main components of this fraction. Carbohydrates represent 46

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to 53% of the yeast composition [7], being represented, on average, by 33% trehalose, 27% glucans, 21% mannan-oligosaccharides and 12% glycogen [6]. Yeast has high concentrations of B vitamins, mainly thiamine, riboflavin, niacin, pantothenic acid, and inositol. Another important component of yeast is lipids (around 1.45%) [7] and nucleotides, represented by nucleic acids [6]. The cell wall is mainly composed of mannoproteins (35–40% of the dry matter of the cell wall) and the inner part is composed of polymers of glucose: β(1,3)-and β(1,6)-glucan (50–65% dry matter of the cell wall) [8].

S. cerevisiae is the most powerful, single-cell eukaryotic system for biological research and industrial applications, due to its remarkable resistance/tolerance to high sugar concentrations and production of several byproducts of commercial interest. *S. cerevisiae* can grow on a simple range of fermentable and non-fermentable carbon sources (mostly six-carbon sugars) [4], and agri-food residues can be used as main carbon and nitrogen sources for yeast growth. When nutrient starvation occurs, sexual cycles can be activated by environmental motivation, and result in the production of four meiotic spores that have two distinct mating types [4].

A mix of commercial or domesticated strains, and/or environmental (or "wild") strains can be used as starters in industrial applications [9]. *S. cerevisiae* shows the potential to fast growth in a culture medium with appropriate oxygen availability and controlled carbon source feeding; on the other hand, it can produce ethanol in anoxic conditions. Indeed, yeasts are widely used in the elaboration of food and feed products (mainly single cell protein or yeast extract, YE), commonly found in the production of fermented foods and beverages, functional foods, food additives (FAs) and ingredients [10]. The fractionation of *S. cerevisiae* cells has been widely applied in the food market to obtain mannoproteins, glucomannans, yeast glycans, yeast protein concentrate, invertase, ergosterol, glutamic acid, peptides, amino acids, and glucans [11, 12]. Other important products such as alcohols, esters, aldehydes and terpenes, volatile compounds, polyphosphate, and antioxidants can be obtained from *S. cerevisiae* and used as FAs. These additives have focused on enhancing mouthfeel, taste, and aroma; reducing sugar, fat, and salt addition to the final product; bringing nutritional advantages to the final product and masking off-note [11, 12]. Indeed, *S. cerevisiae* products are recognized as safe by the FDA (Food and Drug Administration) [13, 14] and by the Regulation (EC) No. 1334/2008 [14] they are animal-free, allergen-free, organic, and a clean label product. Figure 7.1 shows some FAs obtained from *S. cerevisiae*.

Around 0.4 million metric tons of yeast biomass are generated each year worldwide [9]. In 2020, the global yeast market was of USD 3.9 billion, and it shows an estimative to reach USD 6.1 billion until 2025, showing a compound annual growth rate of 9.6% during this period [15]. This market growth may be related to the fact that consumers are prone to try new products such as plant-based meat since the concern with environmental issues opens the field for new sources of protein and healthier meat products that say they are eco-friendly. In this regard, these yeast additives can be used to improve meat flavor and nutrients for this large and growing market of meat analogs.

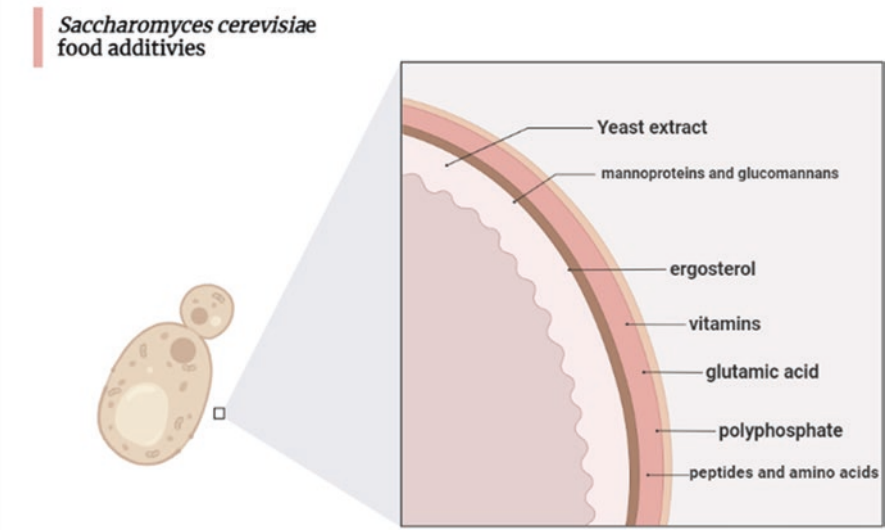

Fig. 7.1 Examples of food additives obtained from *S. cerevisiae*. (Created in BioRender)

This chapter reviews the state of the art regarding *S. cerevisiae* production system and culture media used for its production as a food additive. Furthermore, this chapter aimed to describe the main application and components of yeast extract and inactive *S. cerevisiae*, umami taste and meaty flavor, cell wall, polyphosphate, ergosterol and future direction to the use of genetically modified organism (GMO) *S. cerevisiae* in the food additive market.

7.2 *Saccharomyces cerevisiae*: Culture Medium and Operation Mode for Products and By-Products Production

S. cerevisiae biomass has a lot of potential for the application in different areas since it is generally considered safe (GRAS, Generally Recognized as Safe) by the FDA and due to the various components obtained through its metabolism, such as fats, carbohydrates, nucleic acids, vitamins, and minerals [16], which are extremely attractive to its application as a food additive. In addition, yeasts can grow fast and consume fewer quantities of substrates to produce higher biomass concentrations when compared to plants or animals. Also, many substrates can be used for their growth, including wastes and residues from different processes, which apart from being a cheap alternative, in this perspective, makes them environment friendly [16].

As aforementioned, different products can be obtained through *S. cerevisiae* biomass production such as the cell itself – as single-cell protein [17], yeast extract [18], ergosterol [19–21], β-carotene [22], β-glucan [8], and others. The culture

medium choice will affect the yeast biomass composition obtained during growth cultivation and it will affect the product's profile [23]. In culture medium definition, it is important that all nutrients are considered safe, so the resulting products' safety is not affected.

In literature, a variety of substrates have been described as possible culture mediums for yeast biomass production (Table 7.1). It is usual to apply synthetic medium for laboratory research since all components and concentrations are known and there is no need for pre-treatment or additional steps for its preparation (upstream) that are usually required by biomasses, such as wastes and residues. In this context, Lavová et al. [19] used a synthetic cultivation medium to produce ergosterol by *S. cerevisiae*. They tested three different yeast strains and *S. cerevisiae* 612 reached the highest ergosterol yield (2.82 mg of ergosterol/g of biomass), which is highlighted in Table 7.1.

Vegetable biomasses also have been applied for cell propagation and consequently, different products. Bioenergy sorghum was used by Cheng et al. [22] to produce β-carotene by an engineered *S. cerevisiae*. The biomass was subjected to hydrothermal pretreatment (190 °C for 10 min) and acid hydrolysis (120 °C, 5 min, 2% sulfuric acid loading) to release fermentable sugars. The fermentation using this high-concentrated hydrolysate resulted in a yield of 7.32 mg of β-carotene/g of biomass. In another research, Tan et al. [20] produced ergosterol from corn steep liquor (a residue from corn mills that is rich in nitrogen) combined with industrial glucose by *S. cerevisiae* (N°. A-3) in a fed-batch strategy. In this study, it was possible to achieve a yield of 1.20 g of ergosterol/L.

Agri-food wastes are very interesting carbon and nitrogen sources to compose *S. cerevisiae* culture medium since they are cheap and promote lower generation of waste and pollutants. The possibility of obtaining high value-added products from those residues is extremely inviting [18]. There are a variety of wastes that can be used as carbon source for *S. cerevisiae* cultivation, such as food peels hydrolysates obtained from a pre-treatment of the respective waste, as studied by Bacha et al. [17] (Table 7.1). The authors used potato, apple, carrot, and orange peels, pretreated with hydrochloric acid (10%), to produce *S. cerevisiae* single cell protein and reached the highest yield of 5.29 g of biomass/40 g of potato peel [17]. Corn straw hydrolysate is a lignocellulosic biomass that has been studied as a cheap carbon source to produce value-added products, as done by Wu et al. [21], that tested ergosterol production by *S. cerevisiae*. The corn straw was pre-treated by steam (at 200 to 240 °C for 15 to 20 min) followed by acid hydrolysis (HCl 1:10 solid: liquid) and after, it was fermented by *S. cerevisiae* in batch mode, resulting in the stability of ergosterol content in yeast cell extraction solution, crystallize ergosterol productivity and the mean extraction yield values are shown to be 2.35, 2.05, 87.24%, respectively.

Agri-food wastes can also be applied to yeast extract (YE) production, as studied by Ganatsios et al. [18]. The authors developed a biorefinery process in which orange wastes were applied as supports for yeast immobilization, as culture medium for the brewing process. After the brewing process, the yeasts cells were used for the alternative yeast extract (AYE) production. Orange pulp was applied for

Table 7.1 Different substrates used to produce *S. cerevisiae* biomass

Yeast strain	Product of interest	Substrate	Substrate Pretreatment	Cultivation Process	Yield	References
S. cerevisiae	Single cell protein	Potato peel	Acid	Cell propagation - Batch	5.29 g of biomass/40 g of peel	[17]
		Apple peel			0.3 g of biomass/40 g of peel	
		Carrot peel			3.61 g of biomass/40 g of peel	
		Orange peel			2.55 g of biomass/40 g of peel	
S. cerevisiae YELB	Ergosterol crystallize	Corn straw hydrolysates	Steam followed by HCl hydrolysis	Cell propagation - Batch	8.84 mg of ergosterol/ g of biomass	[21]
S. cerevisiae AXAZ-1 immobilized in orange pulp	Yeast Extract	Citrus molasses and orange juice	Separation of orange pulp (immobilization) and juice (carbon source)	Brewing - Batch	not shown	[18]
S. cerevisiae strain 612	Ergosterol	Yeast Peptone Dextrose (YPD) medium	Ni	Cell propagation - Batch	2.82 mg of ergosterol/g of biomass	[19]
Engineered *S. cerevisiae*	β-carotene	Bioenergy sorghum hydrolysate	Hydrothermal pretreatment and dilute acid hydrolysis	Cell propagation - Batch	7.32 mg of β-carotene/g of biomass	[22]
S. cerevisiae (N°. A-3)	Ergosterol	Corn steep liquor + industrial glucose	Ni	Cell propagation - Fed-batch	1.20 g of esterol/L of medium	[20]
S. cerevisiae var. *boulardii*	β(1,3)/(1,6)-glucan	Deproteinated potato juice water supplemented with glycerol (10%)	Ni	Cell propagation - Batch (pH 5)	22.80 g of β-glucan/ 100 g of cell wall preparation	[8]
S. cerevisiae R9					20.10 g of β-glucan/ 100 g of cell wall preparation	
S. cerevisiae 102					16.90 g of β-glucan/ 100 g of cell wall preparation	

Ni Not informed

immobilization, forming an immobilized cell biocatalyst (CWBB), which was used for beer fermentation, being citrus molasses and orange juice (resulting from the orange pulp preparation) the carbon source. Then, CWBB went through autolysis for AYE production (see Sect. 7.3.1). Bzducha-Wróbel et al. [8] focused on the biosynthesis of functional polysaccharides (β (1,3)/(1,6)-glucan and/or mannoproteins) by different strains of *S. cerevisiae* (*S. cerevisiae* var. *boulardii* PAN, *S. cerevisiae* R9, *S. cerevisiae* 102), using deproteinated potato juice water supplemented with glycerol and batch operation mode. The authors tested different glycerol concentrations (5, 10, 15, 20, 25%) and pH values (4.0, 5.0, 7.0) and some of the best results are highlighted in Table 7.1. For 10% of glycerol supplementation and pH 5.0, the strains were able to produce 22.80, 20.1 and 16.9 g of β-glucan/ 100 g of cell wall preparation, respectively.

The application of the yeast's biomass as a food additive requires the large-scale propagation of the cells, to obtain high cell densities or the industrial brewing process yeast can be used [18], which is usually discharged or applied to animal feed. The strategies to produce large amounts of YE are explained in Sect. 7.3.1.

Industrially, beet or sugarcane molasses are the main substrates used for *S. cerevisiae*, since they have large amounts of sugars and nutrients, and are cheap [9, 24]. As previously mentioned, lignocellulosic biomasses and agri-food wastes are a great option of carbon source for *S. cerevisiae*; however, unlike beet or sugarcane molasses, they do not present all nutrients required by the yeast's metabolism. In this sense, there is the need to supplement these alternative sources with urea or ammonium salts, to overcome nitrogen deficiency. Also, other nutrients can be added to these culture media to provide high cell densities, such as phosphate salt, magnesium salt, thiamine, pantothenate and biotin [9].

Regarding operation mode for *S. cerevisiae* cultivation, batch is the most used for small scale processes. However, in order to provide proper conditions for higher cell accumulation at large scale, the process is divided into two operation modes, batch and fed-batch (Fig. 7.2, process B). It is important to highlight that *S. cerevisiae* is widely used for ethanol production in mills, and the focus on that operation unit is different from a specialized protocol for yeast propagation, where the objective is to use yeast for cell biomass production. Since *S. cerevisiae* is a Crabtree yeast, different strategies can be applied for different final product production: ethanol or yeasts. In the last one, an inoculum phase is the first step for high sugar concentration adaptation, that is usually performed in a flask – containing the proper substrate and the *S. cerevisiae* strain – that is used to start the batch step, followed by fed-batch phase [25]. Batch fermentation is extremely important for accumulating different metabolites that are used during fed-batch, and pH must be around 4.5 to 5.0 [24, 25]. During the batch phase, aeration and temperature are the two parameters controlled. Generally, growth cultivations are associated with continuous aeration, but Maemura et al. [26] suggested that a nearly short aeration period is enough during batch phase, and consequently, a fermentative metabolism is prevalent [9]. Oxygen is needed because of the energy requirements for cell growth and multiplication [27]. When the carbon source is depleted in the batch phase, the low oxygen content present in the medium oxidizes the ethanol, which promotes the shift in metabolism from

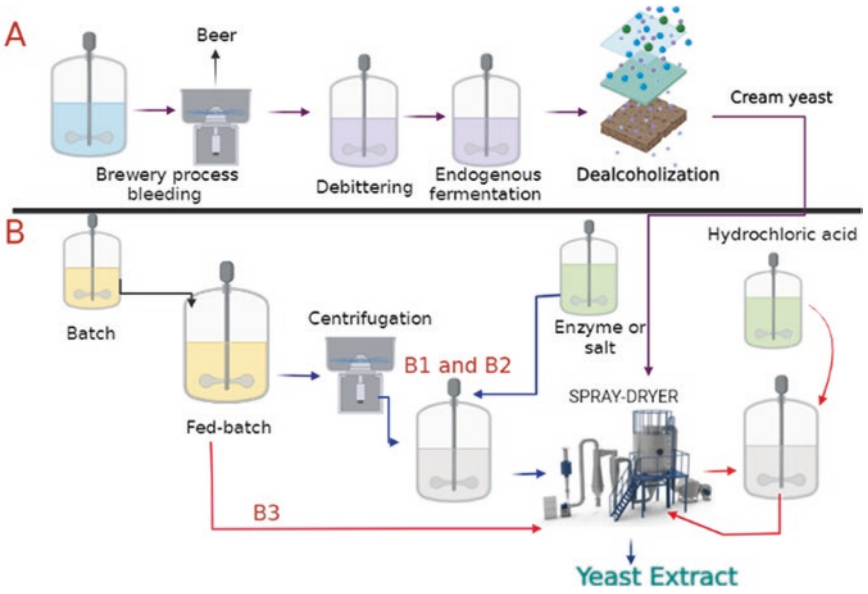

Fig. 7.2 Techniques applied to produce yeast extract. (A) Brewery process bleeding followed by debittering, endogenous fermentation, dealcoholization and drying (purple arrows), and (B) *S. cerevisiae* propagation at controlled carbon feeding rate followed by centrifugation (black arrows), (B1) plasmolysis with salt and ethyl acetate and drying; (Blue arrows) (B2) autolysis with enzymes and drying (blue arrows); and (B3) acid hydrolysis with hydrochloric acid and drying (red arrows). Created in BioRender

fermentation to respiration and eradicates ethanol from broth [24]. The fed-batch begins after the ethanol consumption at the end of the batch phase, and this operation mode allows the adding of nutrients as required by the yeast (Fig. 7.2, process B). This condition promotes higher cell accumulation and consequently allows the process to follow in a sugar-limited cultivation combined with oxygen presence, that promotes respiratory growth for the yeast. Sugar limitation is important, since high sugar concentration combined with high oxygenation can result in the Crabtree effect [24, 27]. *S. cerevisiae* exhibits the Crabtree effect when sugar concentrations are high, even under aerobic conditions [28, 29]. When the Crabtree effect is happening, enzymes involved in the respiration metabolism are inhibited and ethanol production escalates, in which fermentation happens under aerobic conditions [27].

After the yeast propagation, it is necessary to harvest them from the broth through centrifugation (Fig. 7.2), which is possible due to the large size of cells [24]. The following procedures depend on the product of interest that will be obtained from the biomass, but usually cell rupture and fractionation are used to enhance the yeast application into food products [7]. However, there is also the possibility to obtain yeasts through brewing (spent yeast) which requires different steps after cell separation (Fig. 7.2, process A). The biomass production during brewing is a consequence from the process itself. The parameters chosen in this case are mainly to guarantee the characteristics from the product of interest, the beer, but they also promote

biomass accumulation, and those cells have a lot of potential in the obtention of yeast extract [18] and other products. After the brewing process some other additional steps are necessary, such as debittering, endogenous fermentation and dealcoholizing [9], that are further discussed in the next topic (Fig. 7.2, process A).

7.3 *S. cerevisiae* Food Additives

7.3.1 Yeast Extract and Inactive Yeasts

YE is obtained when the cell wall is destructed and removed since the soluble internal substances are the ones applied for it. The different cell components allow YE to be applied in a variety of fields [30]. Flavor attributes in broths, soups, and condiments can be obtained by YE, a common food additive that can deliver an umami flavor and meaty aroma. Amino acids and nucleotides are the main compounds in *S. cerevisiae* that are responsible for flavor assignment [31]. YE also played an important role in the food industry as a protein enricher, due to its high protein content, high concentration of B complex vitamins [9] and excellent amino acid balance. Powder or paste are the commercially available form of YE. Pastes of YE are used in both vegetarian and conventional diets as additives for soups or ready-to-eat meals [32].

S. cerevisiae cultivation for YE production can be performed by specific industrial protocols or using cream yeast obtained from the brewery process bleeding [9], defined as spent yeast. As previously mentioned, when the last one is used, some additional steps can be carried out before yeast lysis, such as debittering, endogenous fermentation and dealcoholization (Fig. 7.2, process A). NaOH solution is generally used to promote the cream yeast debittering, with the aim to reduce bitter taste (iso-alpha acids, resins and hop tannins) [6]. Endogenous fermentation is characterized by the consumption of storage carbohydrates and increase of protein content in the yeast, mediated by carbon starvation and aeration in bioreactors. Next, dealcoholization is preceded by distillation, reverse osmosis or washing [33]. On the other hand, optimized industrial yeast production is based on a fed-batch process using sugarcane molasses or corn hydrolysate or beet syrup. According to Pérez-Torrado et al. [9], the process is characterized by a sequence of increased volume bioreactors with the aim to attain high cell densities (Fig. 7.2, process B), as detailed discussed in Sect. 7.2.

There are three main processes for the lysis of *S. cerevisiae* to obtain YE: (i) autolysis, (ii) plasmolysis, and (iii) hydrolysis (Fig. 7.2). Plasmolysis is a technique based on yeast cells' osmotic stress caused by compounds such as salt and ethyl acetate added to the surrounding medium, causing cell lysis by osmotic shock (B1 in Fig. 7.2). High salt content is observed in the final product by this technique, which limits its use [34].

The most efficient technique for yeast solubilization is acid hydrolysis; on the other hand, it is also the least applied for commercial scale production (B3 in Fig. 7.2), due to high the salt content in the final product. After yeast cell production

and drying, a concentration of 65–85% of yeast is subjected to hydrochloric acid treatment (pH of 1–5, at 60–100 °C), with the aim to degrade proteins, carbohydrates, and nucleic acids. Next, sodium hydroxide is used to neutralize (to pH 5–6) the acidic material, and then it is filtered, concentrated, or dried at 5% moisture content [6].

Autolysis (B2 in Fig. 7.2) can be described by autodigestion, and cellular elements are solubilized by enzymes (proteases, glucanases, nucleases, or phosphodiesterase) present inside the cell itself [35] or added to the process. pH, temperature, and time are controlled, and certain agents are added to the process to enhance autolysis. Yeast extract is the soluble part of the final product and relates to the protein concentrate, that is released naturally by the cell after degradation of the intracellular content [36]. Ultrasonic disruption, electric pulses, and high-pressure homogenization can be used before autolysis to increase the extraction yield of yeast intracellular products [31]. As mentioned above, YE has a wide range of flavors varying according to the methods used for their production and it is influenced by the relations among amino acids, peptides, nucleotides, and carbohydrates present in the extracts. The work of In et al. [37] investigated amino the acids composition of a YE from a spent brewer after a treatment (enzymatic, heating and debittering) and according to the results, the amino acids content in YE was around (%) Glu 11.6, Asp 4.0, Phe 13.0, Lys 8.0, Leu 17.0, Thr 3.0, Val 8.0, Met 2.0, Arg 3.0, Ala 12.0, Tyr 3.8.

According to Biospringer [12], YE has been used in snacks (28%), prepared meals as Bolognese spaghetti (23%), sauce (16%), fish based prepared meals (12%), and soups (10%) as a food additive.

Other important products that have been used as FAs to enhance or preserve wine aromatic composition and/or improve desired mouthfeel properties are inactive yeasts (IY). To produce them, the yeast cream (obtained from A or B in Fig. 7.2) is pasteurized and sterilized, to inactivate yeasts, and after they are subjected to a spray dryer. Nutritional properties are maintained after this procedure [35], such as cheese-type flavor, protein and vitamins source, and peptide glutathione, which helps break down the gluten matrix and reduce mix times in bakery products. In a study carried out by Pozo-Bayón et al. [36] different IY preparation were used to wine production, and according to the results, some compounds like peptides, aspartic and glutamic acid, free amino acids, α-alanine, γ-aminobutyric acid, and other were released by IY to wine.

7.3.2 Salty Taste, Umami Taste and Meaty Flavor From Yeast Extract

The tongue and nose recognize the taste and flavors of what we eat or drink, and they send the information to our brain. There are five basic tastes known: saltiness, sweetness, bitterness, sourness, and umami [6]. Though the umami featured monosodium glutamate (MSG), 5'-guanosine monophosphate (5'-GMP), and inosine

5'-monophosphate (5'-IMP) are also naturally present in many foodstuffs [38] and are also related to the umami taste. These compounds can be extracted from tomato, asparagus, and seaweeds [38] to enhance umami taste; however, *S. cerevisiae* stands out as an excellent umami taste provider, due to the possibility of large volume and controlled production [10] and high umami content. When *S. cerevisiae* is used as a source of umami taste, YE can be fractionated to obtain these compounds [39]. MSG is obtained from yeast hydrolysis (see Sect. 7.3.1), in association with its oligomers and other amino acids that have umami characteristics [40]. The content of glutamate and other amino acids or oligopeptides with umami properties is related to the YE production process selected [32]. The umami taste and meaty flavor can be produced by thermally treated YE since thermal treatment accelerates hydrolysis (taste-active oligopeptides and peptides) and causes the pyrolysis, generating new aromas.

5'-GMP and 5'-IMP can be obtained from *S. cerevisiae* RNA hydrolysis. YE has high RNA content (2.5–15%), and the conventional method for its extraction is by YE treatment with a solution based on hot alkaline sodium chloride (5–12%) [6]. Centrifugation is used to separate the soluble fraction and by the addition of an acid or ethanol, the RNA is precipitated. Obtained RNA can be spray-dried for use as a substrate to produce 5'-nucleotides. During this process, RNA is degraded releasing 2'-, 3'- ribonucleotides (with no flavoring characteristics and are of little commercial interest); associated with 5'- ribonucleotides obtained at high yields (>86%) [39]. The last one is a high value-added molecule used in the foodstuffs. Due to the high by-product releasing during acid hydrolysis, the production of 5'-nucleotides is preferred by enzymatic hydrolysis by proteases, 5'-phosphodiesterase, and nucleases [39].

The limit of MSG addition is 100–300 ppm, on the other hand, 5'- GMP and 5'-IMP stands at 35 and 120 ppm (concentrations in aqueous solution), respectively [32] The association of 5'-nucleotides and umami amino acids in foodstuffs as additives results in low salt, sugar, and fat addition to the final food product ('light' foodstuffs). In 2017, safe levels for glutamate FAs were recommended by the FDA [29].

Soups and sauces for reheating ready-to-eat dinner dishes, meat and mushroom fillings, cold meats, pate, savory snacks, and a range of food concentrates are conventional and organic food that has manufactured with these yeast formulations (rich in MSG, 5'-IMP and 5'-GMP) [32].

7.3.3 Cell Wall Components

S. cerevisiae cell wall is approximately 70–100 nm thick [41] and contains three major constituents: glucan (glucose polysaccharide), mannan (mannose polysaccharide), and a protein fraction [42]. The yeast cell wall plays an important role as a physical barrier to protect cells against stress factors in the environment [43]. The cell wall structure is composed of two layers that have different chemical

compositions. The first one, the inner part of the cell wall, is built with glucose polymers: β(1, 3) and β(1, 6)-glucan and the main structural components of the organelle (50–65% dry substance of the wall). The other layer is composed mainly of mannoproteins, constituting about 35–40% of the dry matter of the yeast cell wall [44, 45].

The separation of these polymers is simple and inexpensive when low yields are required, however, it is difficult and expensive to obtain more than 65% pure fractions. Because of that, these components are produced and sold at low levels of purity. Their largest commercial application has been as animal feed nutritional supplements, but yeast cell wall is known as a food grade ingredient for humans.

The thickness of β-glucan and mannoprotein layers are dependent on the strain of yeast and growth conditions (see Sect. 7.2), like the kind and availability of nutrients in the growth medium, temperature, pH, aeration and duration of breeding but also the phase of the cell cycle [41, 44, 46]. Under conditions of environmental stress, yeast activates mechanisms responsible for cell survival. Modification of the cell wall structure is one of the strategies for yeast survival [43, 47]. Mannoproteins play an important protective function under the stress, since it connects to the loss of water [43], and creates a permeability barrier for macromolecules [48]. β(1,3)-Glucan functions as the rigid structural skeleton of the wall, to which β(1,6)-glucan, mannoproteins and chitin are cross-linked. β(1,3)-glucan protects yeasts against adverse outer conditions to the cell and it is responsible for osmotic stability [46].

Moreover, cell walls are used as a novel coating for the encapsulation of different materials in the food industry. For example, it has been shown that flavors encapsulated in empty yeast cells leaked out only at temperatures higher than 243 °C [49]. By their phospholipid membranes, yeast cells can behave as liposomes and have been used for the encapsulation of both hydrophobic and hydrophilic molecules [50].

7.3.3.1 β-glucan

As the predominant polysaccharide in the *S. cerevisiae* cell wall, β-Glucan have exhibited multifunctional features, including wound healing, antimicrobial, immunomodulation (Epicor™), probiotic, antioxidant, and anticholesterolemic effects, justifying its approval as a dietary supplement by health regulatory agencies [32, 51, 52]. β-Glucan extracted from spent yest exhibits promising *in vitro* antioxidant activity, protection against oxidative damage in calf thymus DNA, and antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus subtilis*, and *Salmonella enterica* serovar Typhi, and other. It also shows prebiotic activity; and it is used as a nondigestible dietary ingredient with a beneficial effect on the host by selectively stimulating the growth of and/or activating the metabolism of health-promoting microorganisms in the gut [16]. Vlassopoulou et al. [53] compiled randomized controlled trials regarding health-promoting effects in humans, triggered by the consumption of β -(1,3 and 1,6)-D-glucans, indicating that the oral administration of this compound from 2.5 to 1000 mg daily for up to 6.5 months led to immune system boosts, minimizing infections, and allergic

symptoms and their incidence. By the reduction of inflammation or the immune reactions (i.e., it exerts a prophylactic effect against common cold infection), an immunomodulatory activity can be induced [16]. In this sense, the β-glucan acceptable daily intake is up to 375 mg/day as food supplements, and up to 600 mg/day as nutritional food [54].

Moreover, β-glucan as a cell wall polysaccharide obtained from the yeast also can be used in food products as an emulsifying stabilizer, or oil-binding, water-holding, and thickening agent [55]. A study by Piotrowska et al. [56] showed that when β-glucan, obtained post-production of spent yeast (Fig. 7.2, process A), is added to yogurt in 0.15 to 0.9% amounts then at 0.3% this does not adversely affect sensory food quality, texture, and stability of the liquid product during storage. A dietary intake of 250 g of this product can provide the body with 0.7 g of β-glucan, affording pro-health benefits and meeting the criteria set for functional foods [57].

In Europe, β-glucan is approved as a novel food ingredient by the Regulation of the European Parliament and Council (EU) 2015/2283 from 25th November 2015 concerning novel foods, amending the Regulation of the European Parliament and of the Council (EU) No 1169/2011 and repealing Regulation (EC) No 258/97 of the European Parliament and Council along with Commission Regulation (EC) No 1852/2001. Thus accordingly, β-glucan from yeasts can be used in dietary supplements, fruit drinks, cereal bars, biscuits, crackers, breakfast cereals, yogurt, chocolate, soup, protein bars and foodstuffs intended for particular nutritional uses, excepting infant formulae. Spent yeast β-glucan is generally recognized as safe (GRAS) by the FDA [58] and Brazilian Health Regulatory Agency (ANVISA) also approved β-glucan as a functional food in 2008 [59].

7.3.3.2 Mannoproteins

Cell wall mannoproteins exhibit many techno-functional properties that make them attractive for food applications. The techno-functional and health properties of cell wall mannoproteins are related to their structural and molecular properties, the monosaccharide composition, molecular weight, and the glycosylation extent [30]. Cell wall mannoproteins are natural emulsifiers in mayonnaise or salad dressing without affecting the sensory attributes since they have shown high emulsifying and stabilizing properties [60–62]. Due to the complexation with phenolic compounds, their techno-functions in the adsorption of ochratoxin A, the inhibition of tartrate salt crystallization, the increase in the growth of malolactic bacteria, the prevention of haze formation and the reinforcement of aromatic components, cell wall mannoproteins have also been extensively used in the enology industry [63]. Mannoproteins stimulate the growth of lactic acid bacteria [64] and inhibit pathogenic bacteria [64] since they showed health promoting benefits.

The isolation of mannoproteins from yeast/yeast cell wall by-products with well-defined structures and glycosylation levels is challenging because of the heterogeneity of their structures [65–67]. Li and Karboune [60] cited that the acid-alkaline method has mainly been used for the isolation of mannoproteins from yeasts. This

study showed that after NaOH (2%, w/v, 2 h) treatment of spent yeasts, the yield, and the purity of the mannoproteins extract were 5.93% and 88.24%, respectively [60]. Heat treatment (for the isolation of non-covalently bound mannoproteins), sodium dodecyl sulfate (SDS) (used for the isolation of mannoproteins that are loosely associated with the cell wall, but SDS can also extract the majority of the plasma membrane proteins from yeasts) and enzymatic method are also used for mannoprotein isolation. The enzymatic method with β-1,3-glucanase was investigated as a potential one for the isolation of the covalently bound cell wall mannoproteins [60].

7.3.4 Ergosterol

Ergosterol ($C_{28}H_{44}O$) is a sterol compound responsible for the cell membrane integrity, permeability, and transport along with the plasma-membrane proteins activity and cellular cycle. Also, it is crucial for Vitamin D2, bile acids and steroid hormones synthesis and during stress adaptation in fermentations [68, 69]. In yeasts, such as *S. cerevisiae*, ergosterol is present in the plasma-membrane as its free form and as fatty acids in lipids. Ergosterol synthesis, because of its asymmetric center present in the molecule, needs complex steps, while having low yield and high cost [70].

This sterol is interesting as a food additive since it can reduce cholesterol absorption in the intestinal tract and to impair its synthesis in the human body. With that, low-density lipoprotein (LDL) cholesterol levels are reduced and consequently the risk of coronary heart failure decreases. In addition, ergosterol has anti-cancer, antiproliferative, anti-inflammatory and antimicrobial properties [71].

One of the ways to produce ergosterol industrially is by *S. cerevisiae* fermentations. Aerobic fermentation is the most charming approach for ergosterol production and *S. cerevisiae* has been applied due to its easier manipulation and the great knowledge of sterol's metabolism [69]. Oxygen concentration was shown to be an important parameter for ergosterol production in either batch or fed-batch fermentations [70]. When oxygen is available, the cells do not assimilate exogenous sterols, but synthesize their own ergosterol to meet their metabolism needs [70].

Ergosterol is produced during cell propagation. After the cultivation step, the cells are usually separated from the broth by centrifugation and a few steps are followed for ergosterol extraction. After centrifugation, the cells are subjected to a solution containing KOH, for saponification by heating and extraction with some type of ether. Lavová et al. [19], studied the disruption of yeasts cells in the suspension of sea sand and alcoholic KOH (10%) after a centrifugation step. After that, saponification by heating (90 °C) and extraction with diethyl ether was performed, followed by evaporation at 50 °C to dryness. Wu et al. [21] used *S. cerevisiae* cells cultured and centrifuged for ergosterol recovery by KOH plus ethanol solution. For saponification, the mixture was heated to 80 °C and then petroleum ether was added to extract the ergosterol.

7.3.5 *Polyphosphate*

Phosphoric acid and carbonates are used as raw materials for polyphosphates (PolyP) synthetic production. PolyP are considered non-toxic to humans [13] and biodegradable, and they are used as a food additive in bacon, ham, fish, poultry, and shellfish [72]. PolyP has a buffering capacity (avoid changing the color in product during storage), antibacterial effect (prolong the product shelf-life), water retaining ability, and stabilize suspensions, emulsions and dispersions, and favoring gel formation and water binding capacity. The acceptable daily intake corresponds to an intake of 2.8 g of phosphorus per day for an average adult weighing 70 kg [73]. PolyP can be produced by *S. cerevisiae* since it has high PolyP content (28%) [74], however, there are no reports on the soluble polyP by *S. cerevisiae* production in a highly scalable biotechnological process [74].

Christ and Blank [75] cultured *S. cerevisiae* VH2.200 (an industrial yeast strain used for yeast extract production and bakery foodstuff) in aerobic and anaerobic conditions by using a culture media containing glucose, mineral salts and vitamins aiming orthophosphate (Pi) starvation followed by Pi feeding protocol for YE production. After YE production, autolysis, plasmolysis and enzymatic hydrolysis with or without prior heat inactivation were applied for YE fractionation. The results showed that aerobic and anaerobic conditions had no difference related to PolyP production, and by the protocol established, around 28% (w/w) polyphosphate (as KPO_3) was attained. Enzymatic hydrolysis was the most promising protocol to biotechnologically produce PolyP-rich YE. According to these authors, PolyP is a promising clean-label food additive and the desired characteristics of this additive include a linear molecular structure, appearance as a dry white water-soluble powder, and a purity comparable with chemically produced polyP, and food-grade quality.

7.4 *S. cerevisiae* as Chassis for Biotechnological Food Additive Production

7.4.1 *Special Requirements to Choose* S. cerevisiae *as the Host Organism*

To select the host organism to produce a heterologous metabolite some general considerations need to be made, such as the available tools for the expression of heterologous proteins (strains, vectors, promoters and signal peptides), genetic information and the availability of classical genetic approaches as well as the accessibility of modern molecular biological tools. In this sense, *S. cerevisiae* is one of the most used model hosts in metabolic engineering. Because it is recognized as safe and it has a high tolerance to high osmotic pressure, low pH, and phage infection, making it advantageous in industrial fermentation. Furthermore, its

engineering and applications are greatly facilitated due to the well-defined physiological information and sophisticated metabolic engineering tools. Fortunately, *S. cerevisiae* has excellent DNA transformation efficiency and high inherent homologous recombination efficiency [76], which facilitates genetic manipulations. Since the yeast was first sequenced in 1996, the genetic toolbox has been under continuous development and the last few years have brought significant progress in this area. These unique skills make *S. cerevisiae* useful for biotechnological purposes, especially food and beverage fermentations.

As already cited, the genome of *S. cerevisiae* was completely sequenced in 1996 [1]. Two years later, a website, Saccharomyces Genome Database (SGD) (http://www.yeastgenome.org/) was created to provide the genomic information, functions, and pathways of the budding yeast [77]. During the next decades the knowledge advantage and the single gene deletion collection, covering 96% of annotated open reading frames (ORFs) of *S. cerevisiae*, was constructed [78]. Many tools for RNA profiling have been developed like microarray, which uses *S. cerevisiae*'s genome plasticity enabled strains to diverge through deletion, duplication, horizontal gene transfer, and introgression upon hybridization, mainly in the chromosomal DNA [2, 79]. *S. cerevisiae* contains extrachromosomal materials, namely the 85.78 kb mitochondrial DNA (mtDNA) and the so called 2 μm circle plasmid with 6.318 kb, located in the nucleus, comprising multiple copies and categorized as facultative, since its absence causes minimal growth rate reduction, and does not affect industrial applications [10, 80].

7.4.2 Genetic Tools and Techniques to Build and Improve Yeast Strains

Many different systems have been developed for the genetic modification of yeasts (Fig. 7.3). Exogenous DNA introduced into the cell by several transformation methods is the most used technique, and it has been a PCR-based gene targeting method. Endogenous DNA can be the source to express enzymes in the genetically engineered microorganism, from the exogenous (called heterologous expression) or by the same organism (e.g., to produce more of an existing enzyme). 'Self-cloning' is obtained when the exogenously DNA is sourced from a closely related microorganism capable of natural DNA exchange (frequently the same genus) [81]. To construct a comprehensive set of gene deletion mutants in *S. cerevisiae* a native double stranded break (DSB) repair system has been used [78, 82]. It is difficult to study the phenotypic effects of gene families in this technique, due to multiple genes need to be deleted in the same background to reveal the phenotype [83]. In this sense, researchers have used a marker recycling method, exploiting site specific recombinase technologies that utilize recombinases. This is particularly useful if retention of the selectable marker in the genome is not desirable, or the same selectable marker is to be used to delete another gene (marker recycling). Cre-loxP-mediated recombinase and *delitto perfetto* are good examples of such a system.

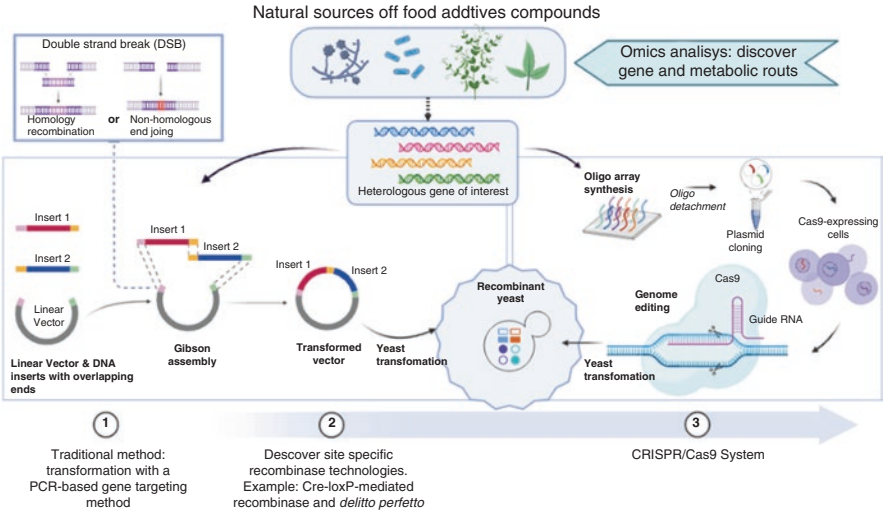

Fig. 7.3 Evolution of methodology yeast genome modification. (1 and 2) Double strand break (DSB) mechanism mediated either by non-homologous end joining (NHEJ) or homologous recombination (HR). To increase the efficiency of genome editing by HR, Cre-*loxP* mediated recombination or in *delitto perfetto* a CORE cassette is used to replace a gene or sequence of interest by HR. (3) CRISPR/Cas9. A plasmid system is used to express the Cas9 endonuclease and guide RNA (gRNA) or, the Cas9 gene is integrated into the yeast genome and the gRNA is delivered on a plasmid. (Created in BioRender)

However, the CRISPR system has modernized the area of precision genome engineering in many organisms, including yeast. The application of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) systems in genome editing is a breakthrough in the development of genetic tools. CRISPR-Cas is a complex of genetic guides (CRISPR sequences) and enzymes (Cas proteins) that together find and edit DNA [84]. The CRISPR/Cas9 system was first introduced into *S. cerevisiae* by DiCarlo et al. [85] in 2013. The CRISPR/Cas9 system allows for highly efficient, multiple and simultaneous, and seamless knock-out or knock-in events.

Due to recent advancements in genome editing methods like CRISPR/Cas and high-throughput omics tools, biotechnological applications of synthetic biology tools including multiplex genome engineering are expanding rapidly and the construction of strategically designed yeast cell factories becomes increasingly possible. The yeast *S. cerevisiae* became an important synthetic biology chassis for high-value metabolite production. Multiplex genome engineering approaches can expedite the construction and fine tuning of effective heterologous pathways in yeast cell factories. Numerous multiplex genome editing techniques have emerged to capitalize on this recently. Coupling the CRISPR/Cas system with traditional yeast multiplex genome integration or donor DNA delivery methods expedites strain development through increased efficiency and accuracy. Novel approaches such as replacing synthetic sequences in the genome along with improved

bioinformatics tools and automation technologies have the potential to further streamline the strain development process. In addition, the techniques discussed to engineer *S. cerevisiae* can be adapted for use in other industrially important yeast species for cell factory development [85].

7.4.3 S. cerevisiae *Recombinant in Additive Food Production*

Several recombinant *S. cerevisiae* strains are reported for food additive production. In Table 7.2 we compiled examples of strains constructed and its application. As we can see, a lot of these strains were modified to produce bioactive compounds, antioxidants originally extracted from plants, like resveratrol [86–91] coumaric acid [92, 93], vitamins [94, 95], or pigments like anthocyanins, β-carotene and lycopene [22, 96–98]. *S. cerevisiae* is reported for pigments as the better host organism for heterologous production of anthocyanins (ACN), because the full-length pathways to most ACN structures require the action of multiple plant enzymes, which are normally difficult to express in bacterial hosts [96, 97, 99].

Also, genome-scale model of *S. cerevisiae* has been used to identify gene disruption strategies for desired phenotype improvements in several studies for successfully predicting metabolic engineering strategies, e.g., for improving the production of succinic acid [100] and vanillin [101], that are FAs with antimicrobial and flavor activity. Other organic acids that act as food preservatives, like lactate/lactic acid and malate, are reported by heterologous production in *S. cerevisiae* [102–105].

The advances in synthetic biology and metabolic engineering had enabled high levels of triacylglycerols (TAG), linolenic, stearidonic and arachidonic acid production in *S. cerevisiae* [76]. One of these approaches provided possible solutions for cocoa butter equivalents (CBEs) production thanks to recent development in phytomics and multi-omics technologies. Biosynthesis of CBE using engineered *S. cerevisiae* is one promising way to satisfy growing CB demand. Efficient genes of GPATs (acyl-CoA:glycerol-sn-3-phosphate acyl-transferase), LPATs (lysophosphatidic acid acyltransferase), and DGATs (acyl-CoA:diacylglycerol acyltransferase) encoding for 1,3-distearoyl-2-oleoyl-glycerol (SOS; C18:0–C18:1–C18:0) production from oil crops can be screened using both computational and experimental approaches. The expression of these lipid biosynthetic genes in *S. cerevisiae* chassis with high-level C18:0- and C18:1-production ability would strongly increase the CBE production. Metabolic engineering and rewiring have enabled turning *S. cerevisiae* from alcoholic fermentation to lipogenesis, and further systems biology, synthetic biology, evolutionary engineering and other advanced systems metabolic engineering strategies might further increase CBE and other lipids production in *S. cerevisiae* [114].

Some strains were modified to enable *S. cerevisiae* to consume different substrates like xylose, the second most abundant renewable sugar in nature, which is, however, not naturally utilized by this yeast. Currently, the production of vitamin A, protopanaxadiol, p-coumaric acid, carotenoid, xylitol, and other natural products

has been achieved in *S. cerevisiae* (Table 7.2) by fermentation on xylose [93, 98, 106]. For instance, a lycopene biosynthetic pathway consisting of *CrtE*, *CrtB*, and *CrtI* was introduced into xylose-fermenting *S. cerevisiae* overexpressing native *XK* and *Sc. stipitis* derived from *XYL1* and *XYL2*. The resultant strain produced 1.6-fold more lycopene using the mixture of glucose and xylose than using glucose alone [98]. Another recombinant strain of xylose metabolizing *S. cerevisiae* was engineered to carry the pathway for p-coumaric acid production through the expression of tyrosine ammonia lyase (*TAL*) and overexpression of some tyrosine biosynthetic pathway genes [93]. This strain produced 242 mg/L of p-coumaric acid from xylose while the titer was only 5.35 mg/L on glucose.

Another recombinant approach is the genetic modification of *S. cerevisiae* to improve fermentative performance in baker and beverage production. This tool can provide different flavors or decrease undesirable compounds in the final product, for example. In this sense, Denby et al. [107] create drop-in brewer's yeast strains capable of biosynthesized monoterpenes that give rise to hoppy flavor in finished beer, without the addition of flavor hops. To achieve this end, they identify genes suitable for monoterpene biosynthesis in yeast; develop methods to overcome the difficulties associated with stable integration of large constructs in industrial strains and sensory analysis performed with beer brewed in pilot industrial fermentations demonstrates that engineered strains confer hoppy flavor to finished beer. Another example of a recombinant approach is the genetic modification of *S. cerevisiae* to improve the fermentative performance of bakers. This process uses different flour additives such as enzymes (amylases, hemicellulases, and proteases) to change and improve dough properties and/or bread quality. Baker's yeast modification with heterologous genes encodes these enzyme expressions and could substitute these flour additive enzymes. To date, there is no yeast strain used in the baking industry, which is genetically modified, despite some studies demonstrating that the application of recombinant DNA technology is a possibility for improved strains suitable for baking [108].

As a robust cell factory, *S. cerevisiae* has been used to produce many chemicals, ranging from bulk chemicals and biofuels to high-value natural products. Several processes that use engineered yeast as the host have been commercialized (Table 7.3), such as resveratrol (Evolva in 2014), vanillin (Evolva and International Flavors & Fragrances in 2014) and stevia (Evolva and Cargill expected in 2016) [117]. Another example of an additive recombinant product commercialized is RealSweet™ Reb M, a bioidentical to stevia's sweetest molecule. This compound is present in the leaves at only very low concentrations (less than 0.1%), making it difficult to isolate using traditional extraction and harvesting processes. But the biotechnological company Amyris creates the pure Reb M molecule through fermentation, by engineering *S. cerevisiae* and fermenting sugarcane, so none of the challenges that exist with leaf purification exist with Amyris' clean chemistry [116].

As previously commented in beer production, another commercial application of *S. cerevisiae* GMO is in wine production where genetic modification can be made to provide different flavors, enhance bioactive compounds, decrease undesirable compounds, or improve fermentation performance. In these bioprocesses, malolactic

Table 7.2 *S. cerevisiae* recombinant strains described in scientific literature for food additives production

Food Additive	Application	Genetic modification	Titer or yield	Reference
Resveratrol	Antioxidant	Insertion of the genes At*PAL2*, At*C4H*, At4*CL2*,Vv*VST1*, ACC, ARO7fbr, ARO4fbr, ARO10Δ, SeACS, AtATR2, ScCYB5	272.6 mg/dm^3	[86]
		Insertion of the genes of *A. thaliana (4CL), V. vinifera (RS)*	5.25 mg/L	[87]
		Insertion of the genes of *Populus trichocarpa X P. deltoides (PAL, CPR), Glycine max (C4H, 4CL), Vitis vinifera (RS)*	0.31 mg/L	[88]
		Insertion of the genes of *Nicotiana tabacum (4CL), Vitis vinifera (RS)*	6 mg/L	[89]
		A. thaliana 4-coumaroyl-coenzyme A ligase (*4CL1*) and the *Vitis vinifera* stilbene synthase (*STS*)	391 mg/L	[90]
		Introduced multiple copies of the genes Ha*TAL*, At*4CL1*, and Vv*VST1* into a strain, over-expressing Sc*ARO4*^{K229L}, Sc*ARO7*^{G141S}, and Sc*ACC1*$^{S659A, S1157A}$.	415.65 mg/L	[91]
		Four heterologous genes: phenylalanine ammonia lyase gene from *Rhodosporidium toruloides*, cinnamic acid 4-hydroxylase and 4-coumarate:coenzyme A ligase genes both from *Arabidopsis thaliana*, and the stilbene synthase gene from *Arachis hypogaea*. Acetyl-CoA carboxylase (*ACC1*) gene was additionally overexpressed in the yeast by replacing the native promoter of the *ACC1* gene with the stronger GAL1 promoter	5.8 mg/L	[110]
Anthocyanin	Antioxidant, pigments	Insertion of the genes (*F3H, ANS*, and *3GT*), the genes of anthocyanin synthesis were incorporated, deleted genes encoding glucosidases, as well as the pathway of phloretic acid synthesis was abolished	202.3 μM	[96]
		Reconstituted the full pathway to the biosynthesis of pelargonidin-331 3-O-glucoside (*P3G*), cyanidin-3-O-glucoside, and delphinidin-3-O-glucoside	Ni	[97]
Vitamin A	Antioxidant	Upper expression: *SsXYL1, SsXYL2, SsXYL3, CrtE/I/YB, Blh, Δpho13, and ΔAld6*	3350 mg/L	[94]
p-Coumaric acid	Antioxidant	2-oxo acid decarboxylase (*ARO10*Δ), Pyruvate decarboxylase (*PDC5*Δ), *Flavobacterium johnsoniae (TAL)*; *S. cerevisiae*: ARO4^{fbr}, ARO7^{fbr}, ARO1, ARO2	1.71 g/dm^3	[92]
		Expression of tyrosine ammonia lyase (*TAL*) and overexpression of some tyrosine biosynthetic pathway genes	242 mg/L from xylose 5.35 mg/L from glucose	[93]

Xylitol	Sweetener food additive	Overexpressing xylose reductase (XR) genes from *Candida tropicalis*, *Pichia stipitis*, *Neurospora crassa*, and an endogenous gene *GRE3*. The gene encoding a xylose specific transporter (*SUT1*) from *P. stipitis* was cloned to improve xylose transport	Volumetric xylitol productivity: 0.28 g/Lh	[107]
		XR from *P. stipitis*	Yield: 1 g/g	[110]
		XR from *P. stipitis*	Yield: 0.96 g/g	[111]
β- carotene	pigment, antioxidant	Upper expression: *SsXYL1(K271N), SsXY2, XKS,Gal2(N376F), xPk, PTA, tHMG1,CrtEBI, Δpho13,* and *ΔAld6*	903 mg/L	[98]
		Heterologous expressed β-carotene biosynthetic pathway consisting of *GGPP* (geranylgeranyl pyrophosphate) synthase (*CrtE*), phytoene desaturase (*CrtI*), bifunctional phytoene synthase and lycopene cyclase (*CrtYB*). Also heterologous xylose assimilation pathway with xylose reductase (*XR*) and xylitol dehydrogenase (*XDH*)	7.32 mg of β-carotene/g of biomass	[112]
Lycopene	Antioxidant	*crtE* and *crtI* from *Xanthophyllomyces dendrorhous* and crtB from *Pantoea agglomerans*. Enzyme engineering of *CrtE* and *CrtB* (delta-integration) Overexpression targets: *OLE1* improves membrane flexibility, and *STB5* involved in NADPH production. Deletion target: *MOT3*, a homolog of *ROX1*	41.8 mg/gDCW	[113]
L-ascorbic acid	Antioxidant	Construction of a biosynthetic pathway utilizing appropriate endogenous and heterologous enzymes (ALO1 and ARA1 from *S. cerevisiae*, *A. thaliana* AGD and *A. thaliana* LGDH)	100 mg/L	[95]
Vanillin	Flavor, antioxidant and antimicrobial	In silico metabolic engineering strategy for identifying target genes (*PDC1* and *GDH1*) involved in product production and toxicity	5-fold increase in free vanillin production	[101]
Succinic acid	Acidulant, surfactant, ion chelator, antibiotics	Deletion of the primary succinate consuming reaction, *Sdh3p*, and interruption of glycolysis derived serine by deletion of 3-phosphoglycerate dehydrogenase, *Ser3p/Ser33p*.	0.90 g/L	[100]
		Redirecting the carbon flux into the glyoxylate cycle (oxidative production) by quadruple gene deletion (*SDH1, SDH2, IDH1*, and *IDP1*)	Titers are 4.8 times higher (3.62 g/L) in comparison to wild-type	[102]
Latic acid/ lactate	Food preservative	Combining target gene integration (six copies of the bovine L-LDH) with adaptive evolution (ethylmethane sulfonate mutagenesis)	122 g/L (chemostat)	[103]

Pyruvate	Flavor agent	Combining pathway engineering (triple gene deletion – *pdc1, pdc5*, and *pdc6*) with a two-stage evolutionary engineering strategy	135 g/L	[104]
Malate	Acidulant and flavor enhancer in food and beverages	Overexpressing pyruvate carboxylase (*PYC2*), cytosolic malate dehydrogenase (*MDH3*) and a malate transporter *SpMAE1* from *Schizosaccharomyces pombe* in an evolved *PDC*-deficient strain	59 g/L	[105]
Cocoa butter equivalents (CBEs)	Substitute in chocolate products	Genes of *GPAT*s, *LPAT*s, and *DGAT*s [1] increasing fatty acids especially C18 compositions via directing metabolic flux toward lipid synthesis; [2] overexpression of acyl-CoA transferases specially for CBE synthesis	Ni	[115]
Flavor compounds	Beer	drop-in brewer's yeast strains capable of biosynthesizing monoterpenes that give rise to hoppy flavor in finished beer, without the addition of flavor hops.	Ni	[107]
Enzymes	Casein: cheese production	Human κ-casein (*Kex-*) into pD1214 plasmid, transform to *E. coli*, Optimize yeast URA3 selection system.	Ni	[116]
	Improved strains suitable for baking	Heterology expression of amylases, hemicellulases, and proteases	Ni	[108]

Ni Not informed

Table 7.3 *S. cerevisiae* recombinant strains approved for industrial and commercial production of food additives

Product	Genetic modification	Application	Company
RealSweet™ Reb M	Add steviol synthesis rout[a]	Sweetener	Amyris SA [116]
Stevia	Add steviol synthesis rout[a]	Sweetener	Evolva and Cargill expected in 2016 [117]
Veri-te™ resveratrol	Add resveratrol synthesis rout[a]	Antioxidant	Evolva in 2020 [117]
Vanillin	Add vanillin synthesis rout[a]	Flavor, antioxidant, and antimicrobial	Evolva and International Flavors & Fragrances in 2014 [117]
Wine	Linear cassette containing the *Schizosaccharomyces pombe* malate permease gene (*mae1*) and the *Oenococcus oeni* malolactic gene (mleA) under control of the *S. cerevisiae* PGK1 promoter and terminator sequences into the URA3 locus of an industrial wine yeast	Wine production	Hunisk et al. [118]

[a]Genes and original organism gene not described at the approved regulation

fermentation is essential for the deacidification of high acid grape must. Husnik et al. [118] constructed a genetically stable industrial strain of *S. cerevisiae* by integrating a linear cassette containing the *Schizosaccharomyces pombe* malate permease gene (*mae1*) and the *Oenococcus oeni* malolactic gene (*mleA*) under control of the *S. cerevisiae* PGK1 promoter and terminator sequences into the URA3 locus of industrial wine yeast. The malolactic yeast strain, ML01, fully decarboxylated 5.5 g/L of malate in *Chardonnay* grape must during the alcoholic fermentation. Analysis of the phenotype, genotype, transcriptome, and proteome revealed that the ML01 yeast is substantially equivalent to the parental industrial wine yeast. The ML01 yeast enjoys 'Generally Regarded as Safe' status from the FDA and is the first genetically enhanced yeast that has been commercialized. Its application will prevent the formation of noxious biogenic amines produced by lactic acid bacteria in wine.

7.4.4 Regulatory Aspects of GMO Use in Food

Organisms with recombinant DNA are referred to as 'genetically modified' (GM) in Europe and as 'genetically engineered' (GE) in Canada and the US. Many regulations make a distinction between food substances that are "derived from" or "produced from" a genetically engineered source. That is an important aspect when talking about the production of FAs by these recombinant microorganisms, because

under these regulations, such as those in the European Union (Regulation (EC) No 1830/2003), the United States (7 CFR 66), and Canada (CAN/CGSB-32.315-2004), food substances "produced with" a GEM (the GEM is essentially a processing aid or incidental additive) do not require to be labeled as GMO, while food substances "derived from/produced from" a GEM (the GEM and/or its genetic material remain present in the food substance) or other genetically engineered source must be labeled as GMO unless they qualify for another exemption (such as processing aid status for enzymes in most jurisdictions or the exemption for highly refined ingredients in the United States National Bioengineered Food Disclosure regulation). This increases the market and applications of these additives that do not face the barrier of a portion of the market that has resistance to consume GMO-labeled products [81].

In the United States, three federal agencies within the U.S. government work together to regulate most GMOs. "GMO" (genetically modified organism) has become the common term consumers and popular media use to describe a plant, animal, or microorganism that has had its genetic material (DNA) altered through a process called genetic engineering. The U.S. Food and Drug Administration (FDA), U.S. Environmental Protection Agency (EPA), and U.S. Department of Agriculture (USDA) ensure that GMOs are safe for human, plant, and animal health. These agencies also monitor the impact of GMOs on the environment.

In European Union (EU), the Novel Food Regulation has been recently amended by three new regulations concerning genetically modified organisms including derived foods and feeds: EC1829/2003 (EC 2003a), 1830/2003 (EC 2003b) and 65/2004 (EC 2004), which define the procedures for authorization, labeling and traceability. Also, Regulation 1829/2003 describes the information to be provided by an applicant seeking authorization to place a product on the market. The product then goes through the approval procedure between the European Food Safety Agency (EFSA) in Brussels, the European Commission and member states. Labeling is mandatory, even if the recombinant DNA or the corresponding protein cannot be detected in the final product. Foods containing GMOs have to be labeled "genetically modified" or "produced from genetically modified (name of the ingredient)." Labeling is not required for foods containing traces of GMOs, which are adventitious and technically unavoidable, in a proportion lower than the threshold of 0.9% of the food ingredients (relation between recombinant and non-recombinant ingredients). Whereas the Novel Food Regulation was based on the principle of evidence, in the sense of mandatory labeling for food products containing more than 1% GMOs, Regulation EC1829/2003 is supported by the principle of application, making the declaration of GMO use during the production of food compulsory, but declaration does not rely on the detection of recombinant DNA or protein in the final product. According to Regulations No. 1830/2003 (EC 2003b) and 65/2004 (EC 2004), GMOs and products derived from GMOs must be traceable during all stages of their placing on the market through the production and distribution chain, in order to facilitate the withdrawal of products when necessary and to facilitate the implementation of risk management measures.

In Brazil the responsible agency is the National Technical Commission for biosecurity (CTNBio), a multidisciplinary collegiate body, created by law n° 11,105

(2005), with the purpose to provide technical advisory support and advice to the Federal Government in formulation, updating and implementation of the National Biosafety Policy related to GMOs. The agency is responsible for the establishment of technical safety standards and technical opinions regarding the protection of human health, living organisms and the environment, for activities involving construction, experimentation, cultivation, handling, transport, commercialization, consumption, storage, release and disposal of GMOs and derivatives. The commercial approval process of genetically modified organisms must follow the recommendations of Normative Resolution CTNBio n°5/2008 (RN5). The list of approved GMOs in Brazil had 43 strains approved for industrial and commercial use, 20 of which are *Saccharomyces cerevisiae* recombinant strains utilized in biofuel ethanol and farnesene, and food additive steviol [119].

All these regulations require to show that the referred food compounds must not: have adverse effects on human and animal health and the environment; mislead the consumer and differ from the food which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for the consumer. Such products must undergo a safety assessment before being placed on the market, including a technical dossier with detailed information concerning results obtained from research and developmental releases in order to evaluate the GMO's impact on human health and the environment [81].

7.5 Conclusions

S. cerevisiae is the most studied yeast, and it has been used since Neolithic times for food and beverage production. The evolution and domestication of this yeast along with industrial application shows the wide range opportunities to its integral use as yeast extract and inactive yeast or fractionated to obtain additives, such as ergosterol, polyphosphate, umami taste, mannoproteins, glucomannan and other. As people present health concerns regarding animal welfare issues, besides aiming for a healthier life, vegan movements are increasing worldwide. As an attempt to overcome this situation, meat industries are developing meat analogues, which avoid animal killing, by using alternative sources such as microbial ingredients, opening the opportunity for *S. cerevisiae* food additive market increase. *S. cerevisiae* has gained attention due to providing flavor and to achieve an end-product that tastes like meat, besides providing high content of protein, peptides, amino acids and vitamins. It also demonstrates the potential for future molecules in the additive food industry since it can be genetically modified to express different enzymes.

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References

1. Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldman H, et al. Life with 6000 genes. Science. 1996;274:546–67.
2. Duan SF, Han PJ, Wang QM, Liu WQ, Shi JY, Li K, et al. The origin and adaptive evolution of domesticated populations of yeast from Far East Asia. Nat Commun. 2018;9(1):2690.
3. McGovern PE, Zhang J, Tang J, Zhang Z, Hall GR, Moreau RA, et al. Fermented beverages of pre-and proto-historic China. Proc Natl Acad Sci U S A. 2004;101:17593–8.
4. Liti G. The natural history of model organisms: the fascinating and secret wildlife of the budding yeast *S. cerevisiae*. elife. 2015;4(4):e05835.
5. Sampaio JP, Gonçalves P. Natural populations of *Saccharomyces kudriavzevii* in Portugal are associated with Oak bark and are sympatric with S. cerevisiae and S. paradoxus. Appl Environ Microbiol. 2008;74:2144–52.
6. Reed G, Nagodawithana TW. Yeast technology, vol. 2. Netherlands: Springer; 1990. p. 1–459.
7. Bertolo AP, Biz AP, Kempka AP, Rigo E, Cavalheiro D. Yeast (*Saccharomyces cerevisiae*): evaluation of cellular disruption processes, chemical composition, functional properties and digestibility. J Food Sci Technol. 2019;56:3697–706.
8. Bzducha-Wróbel A, Błażejak S, Kieliszek M, Pobiega K, Falana K, Janowicz M. Modification of the cell wall structure of *Saccharomyces cerevisiae* strains during cultivation on waste potato juice water and glycerol towards biosynthesis of functional polysaccharides. J Biotechnol. 2018;281:1–10.
9. Pérez-Torrado R, Gamero E, Gómez-Pastor R, Garre E, Aranda A, Matallana E. Yeast biomass, an optimised product with myriad applications in the food industry. Trends Food Sci Technol, Elsevier Ltd. 2015;46:167–75.
10. Parapouli M, Vasileiadis A, Afendra AS, Hatziloukas E. *Saccharomyces cerevisiae* and its industrial applications. AIMS Microbiol. 2020;6:1–31.
11. Biorigen. Biorigen - Arte em Ingredientes Naturais. 2022. Available from: https://www.biorigin.net/biorigin/index.php/pt/
12. Biospringer. Yeast extract is an ingredient on the rise. 2022. Available from: https://biospringer.com/en/yeast-extract-an-ingredient-on-the-rise/
13. FDA. Food Additive Status List. 2022.
14. FAO. Regulation (EC) No. 1334/2008 of the European Parliament and of the Council on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No. 1601/91, Regulations (EC) No. 2232/96 and (EC) No. 110/2008 and Directive 2000/13/EC. 2008
15. Markets and Markets TM. Yeast Market by Type (Baker's Yeast, Brewer's Yeast, Wine Yeast, Probiotics Yeast), Form (Active, Instant, Fresh), Genus (*Saccharomyces, Kluyveromyces*), Application (Food, Feed), and Region - Global Forecast to 2025. 2022
16. Jach ME, Serefko A. Nutritional yeast biomass: characterization and application. Diet Microbiome. Health Elsevier. 2018;1:237–70.
17. Bacha U, Nasir M, Khalique A, Anjum AA, Jabbar MA. Comparative assessment of various agro-industrial wastes for *Saccharomyces cerevisiae* biomass production and its quality evaluation as single cell protein. J Anim Plant Sci. 2011;21:2011.
18. Ganatsios V, Terpou A, Bekatorou A, Plessas S, Koutinas AA, Koutinas AA, et al. Refining Citrus wastes: from discarded oranges to efficient brewing biocatalyst, aromatic beer, and alternative yeast extract production. Beverages. 2021;7:1–13.
19. Lavová B, Hároniková A, Márová I, Urminská D, Lavová IB. Production of ergosterol by *Saccharomyces cerevisiae*. J Microbiol. 2013;2:1934–40.
20. Tan T, Zhang M, Gao H. Ergosterol production by fed-batch fermentation of *Saccharomyces cerevisiae*. Enzyme Microbial Technol Elsevier Inc. 2003;33:366–70.
21. Wu H, Li Y, Song G, Xue D. Producing ergosterol from corn straw hydrolysates using *Saccharomyces cerevisiae*. Afr J Biotechnol. 2012;11(50):11160–67

22. Cheng MH, Sun L, Jin YS, Dien B, Singh V. Production of xylose enriched hydrolysate from bioenergy sorghum and its conversion to β-carotene using an engineered *Saccharomyces cerevisiae*. Bioresour Technol. 2020;308:1–18.
23. Sadowska A, Dybkowska E, Swiderski F. Spent yeast as natural source of functional food additives. RoczPanstw Zakl Hig. 2018;68:115–21.
24. Gómez-Pastor R, Pérez-Torrado R, Garre E, Matallana E. Recent advances in yeast biomass production. Biomass - Detect Product Usage. 2011;1:201–22.
25. Pereira PR, Freitas CS, Paschoalin VMF. Saccharomyces cerevisiae biomass as a source of next-generation food preservatives: evaluating potential proteins as a source of antimicrobial peptides. Compr Rev Food Sci Food Saf. 2021;20:4450–79.
26. Maemura H, Morimura S, Kida K. Effects of aeration during the cultivation of pitching yeast on its characteristics during the subsequent fermentation of wort. J Inst Brew. 1998;104:207–11.
27. de Andrade APC, da Silva HL, Pinto GAS. Yeast biomass production with potential for biological control: process strategies for increasing yield. Res Soc Dev. 2020;9(4):1515–24
28. Agbogbo FK, Coward-Kelly G. Cellulosic ethanol production using the naturally occurring xylose-fermenting yeast, *Pichia stipitis*. Biotechnol Lett. 2008;30:1515–24.
29. Bonan CIDG, Biazi LE, Dionísio SR, Soares LB, Tramontina R, Sousa AS, et al. Redox potential as a key parameter for monitoring and optimization of xylose fermentation with yeast *Spathaspora passalidarum* under limited-oxygen conditions. Bioprocess Biosyst Eng. 2020;43:1509–19.
30. Jacob FF, Striegel L, Rychlik M, Hutzler M, Methner FJ. Yeast extract production using spent yeast from beer manufacture: influence of industrially applicable disruption methods on selected substance groups with biotechnological relevance. Eur Food Res Technol. 2019;245:1169–82.
31. Dimopoulos G, Stefanou N, Andreou V, Taoukis P. Effect of pulsed electric fields on the production of yeast extract by autolysis. Innov Food Sci Emerg Technol. 2018;48:287–95.
32. Rakowska R, Sadowska A, Dybkowska E, Swiderski F. Spent yeast as natural source of functional food additives. RoczPanstw Zakl Hig. 2017;68:115–21.
33. Jackson RS. Post fermentation treatments and related topics. Wine Sci. 2008:418–519.
34. Breddam K, Beenfeldt T. Applied microbiology biotechnology acceleration of yeast autolysis by chemical methods for production of intracellular enzymes. Appl Microbiol Biotechnol. 1991;35:323–9.
35. Šuklje K, Antalick G, Buica A, Coetzee ZA, Brand J, Schmidtke LM, et al. Inactive dry yeast application on grapes modify Sauvignon Blanc wine aroma. Food Chem. 2016;197:1073–84.
36. Pozo-Bayón MÁ, Andujar-Ortiz I, Alcaide-Hidalgo JM, Martín-Álvarez PJ, Moreno-Arribas MV. Characterization of commercial inactive dry yeast preparations for enological use based on their ability to release soluble compounds and their behavior toward aroma compounds in model wines. J Agric Food Chem. 2009;57:10784–92.
37. In M, Kim DC, Chae HJ. Downstream process for the production of yeast extract using Brewer's yeast cells. Biotechnol Bioprocess Eng. 2005;10:85–90.
38. Ninomiya K. Science of umami taste: adaptation to gastronomic culture. Flavour. 2015;4(13):1–5
39. Alves EM, de Souza JF, de Oliva NP. Advances in yeast autolysis technology - a faster and safer new bioprocess. Brazil J Food Technol. 2021;25:e2020249.
40. Alim A, Yang C, Song H, Liu Y, Zou T, Zhang Y, et al. The behavior of umami components in thermally treated yeast extract. Food Res Int. 2019;120:534–43.
41. Osumi M. The ultrastructure structure and. Micron. 1998;29:207–33.
42. Normand V, Dardelle G, Bouquerand PE, Nicolas L, Johnston DJ. Flavor encapsulation in yeasts: limonene used as a model system for characterization of the release mechanism. J Agric Food Chem. 2005;53:7532–43.

43. Borovikova D, Teparić R, Mrša V, Rapoport A. Anhydrobiosis in yeast: cell wall mannoproteins are important for yeast *Saccharomyces cerevisiae* resistance to dehydration. Yeast. 2016;33:347–53.
44. Klis FM, Mol P, Hellingwerf K, Brul S. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. FEMS Microbiol Rev. 2002;26:239–56.
45. Varelas V, Liouni M, Calokerinos AC, Nerantzis ET. An evaluation study of different methods for the production of β-D-glucan from yeast biomass. Drug Test Anal John Wiley and Sons Ltd. 2016;8:47–56.
46. Dalonso N, Goldman GH, Gern RMM. β-(1→3),(1→6)-Glucans: medicinal activities, characterization, biosynthesis and new horizons. Appl Microbiol Biotechnol. 2015;99:7893–906.
47. Xu S, Zhang GY, Zhang H, Kitajima T, Nakanishi H, Gao XD. Effects of Rho1, a small GTPase on the production of recombinant glycoproteins in *Saccharomyces cerevisiae*. Microb Cell Factories. 2016;15:179.
48. Kollár R, Reinhold BB, PetráKová E, Yeh HJC, Ashwell G, Drgonová J, et al. Architecture of the yeast cell wall - beta-1,6-glucan interconnects mannoprotein, beta-1,3-glucan, and chitin. J Biol Chem. 1997;272:17762–75.
49. Dardelle G, Normand V, Steenhoudt M, Bouquerand PE, Chevalier M, Baumgartner P. Flavour-encapsulation and flavour-release performances of a commercial yeast-based delivery system. Food Hydrocoll. 2007;21:953–60.
50. Shi G, Rao L, Yu H, Xiang H, Yang H, Ji R. Stabilization and encapsulation of photosensitive resveratrol within yeast cell. Int J Pharm. 2008;349:83–93.
51. Rajarajaran A, Dakshanamoorthy A. Beta-Glucans: a biomimetic approach for reducing chronicity in delayed wound healing. J Dermatol Skin Sci. 2020;2:16–21.
52. Vetvicka V, Vetvickova J. Effects of yeast-derived β-glucans on blood cholesterol and macrophage functionality Glucans, blood cholesterol, and macrophage function V. Vetvicka and J. Vetvickova. J Immunotoxicol. 2009;6:30–5.
53. Vlassopoulou M, Yannakoulia M, Pletsa V, Zervakis GI, Kyriacou A. Effects of fungal beta-glucans on health-a systematic review of randomized controlled trials. Food Funct. Royal Society of Chemistry. 2021;12:3366–80.
54. EFSA. Scientific opinion on the safety of 'yeast beta-glucans' as a novel food ingredient. EFSA J. 2011;9(5):2137
55. Thammakiti S, Suphantharika M, Phaesuwan T, Verduyn C. Preparation of spent brewer's yeast b-glucans for potential applications in the food industry. Int J Food Sci Technol. 2004;39:21–9.
56. Piotrowska M, Masek A. Saccharomyces cerevisiae cell wall components as tools for ochratoxin A decontamination. Toxins. 2015;7:1151–62.
57. Waszkiewicz-Robak B, Bartnikowska E. Effects of spent brewer's yeast and biological β-glucans on selected parameters of lipid metabolism in blood and liver in rats. J Anim Feed Sci. 2009;18:699–708.
58. FDA. GRAS Notice 000239: Yeast beta-glucan. 2008
59. Brazil. National Health Surveillance Agency (ANVISA). IN vol:1–30, n 28, 26/07/2018. 2018.
60. Li J, Karboune S. A comparative study for the isolation and characterization of mannoproteins from Saccharomyces cerevisiae yeast cell wall. Int J Biol Macromol. 2018;119:654–61.
61. de Melo ANF, de Souza EL, da Silva Araujo VB, Magnani M. Stability, nutritional and sensory characteristics of French salad dressing made with mannoprotein from spent brewer's yeast. LWT. 2015;62:771–4.
62. da Silva Araújo VB, de Melo ANF, Costa AG, Castro-Gomez RH, Madruga MS, de Souza EL, et al. Followed extraction of β-glucan and mannoprotein from spent brewer's yeast (*Saccharomyces uvarum*) and application of the obtained mannoprotein as a stabilizer in mayonnaise. Innov Food Sci Emerg Technol. 2014;23:164–70.
63. Caridi A. Enological functions of parietal yeast mannoproteins. Anton Leeuw Int J Gen Mol Microbiol. 2006;89:417–22.

64. Ganan M, Carrascosa AV, de Pascual-Teresa S, Martinez-Rodriguez AJ. Effect of Mannoproteins on the growth, gastrointestinal viability, and adherence to Caco-2 cells of lactic acid bacteria. J Food Sci. 2012;77(3):M176.
65. Orlean P. Architecture and biosynthesis of the *Saccharomyces cerevisiae* cell wall. Genetics. 2012;192:775–818.
66. Liu HZ, Liu L, Hui H, Wang Q. Structural characterization and antineoplastic activity of *Saccharomyces cerevisiae* mannoprotein. Int J Food Prop. 2015;18:359–71.
67. de Nobel JG, Klis FM, Priem J, Munnik T, van den Ende H. The glucanase-soluble mannoproteins limit cell wall porosity in *Saccharomyces cerevisiae*. Yeast. 1990;6:491–9.
68. Jordá T, Puig S. Regulation of ergosterol biosynthesis in *Saccharomyces cerevisiae*. Genes MDPI AG. 2020;11:1–18.
69. Hu Y, Zhu Z, Nielsen J, Siewers V. Engineering *Saccharomyces cerevisiae* cells for production of fatty acid-derived biofuels and chemicals. Open Biol. 2019;9(5):190049.
70. Blaga AC, Ciobanu C, Caşcaval D, Galaction AI. Enhancement of ergosterol production by *Saccharomyces cerevisiae* in batch and fed-batch fermentation processes using n-dodecane as oxygen-vector. Biochem Eng J. 2018;131:70–6.
71. Corrêa RCG, Barros L, Fernandes Â, Sokovic M, Bracht A, Peralta RM, et al. A natural food ingredient based on ergosterol: optimization of the extraction from: *Agaricus blazei*, evaluation of bioactive properties and incorporation in yogurts. Food Funct. 2018;9:1465–74.
72. Kulakovskaya TV, Vagabov VM, Kulaev IS. Inorganic polyphosphate in industry, agriculture and medicine: modern state and outlook. Process Biochem. 2012;47:1–10.
73. EFSA. EFSA issues new advice on phosphates. 2019.
74. Christ JJ, Blank LM. *Saccharomyces cerevisiae* containing 28% polyphosphate and production of a polyphosphate-rich yeast extract thereof. FEMS Yeast Res. 2019;19(3):foz011.
75. Christ JJ, Smith SA, Willbold S, Morrissey JH, Blank LM. Biotechnological synthesis of water-soluble food-grade polyphosphate with *Saccharomyces cerevisiae*. Biotechnol Bioeng. 2020;117:2089–99.
76. Li M, Borodina I. Application of synthetic biology for the production of chemicals in yeast *Saccharomyces cerevisiae*. FEMS Yeast Res. 2015;15:1–12.
77. Cherry JM, Adler C, Ball C, Chervitz SA, Dwight SS, Hester ET, et al. SGD: *Saccharomyces* genome database. Nucleic Acids Res. 1998;26:73–9.
78. Giaever G, Chu AM, Ni L, Connelly C, Riles L, Véronneau S, et al. Functional profiling of the *Saccharomyces cerevisiae* genome. Nature. 2002;418:387–91.
79. Legras JL, Galeote V, Bigey F, Camarasa C, Marsit S, Nidelet T, et al. Adaptation of *S. cerevisiae* to fermented food environments reveals remarkable genome plasticity and the footprints of domestication. Mol Biol Evol. 2018;35:1712–27.
80. Rizvi SMA, Prajapati HK, Ghosh SK. The 2 micron plasmid: a selfish genetic element with an optimized survival strategy within *Saccharomyces cerevisiae*. Curr Genet. Springer Verlag. 2018;64:25–42.
81. Hanlon P, Sewalt V. GEMs: genetically engineered microorganisms and the regulatory oversight of their uses in modern food production. Crit Rev Food Sci Nutr. Bellwether Publishing, Ltd. 2021;61:959–70.
82. Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, et al. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science. 1999;285:901–6.
83. Delneri D, Gardner DCJ, Bruschi C, v, Oliver SG. Disruption of seven hypothetical aryl alcohol dehydrogenase genes from *Saccharomyces cerevisiae* and construction of a multiple Knock-out strain. Yeast. 1999;15:1681–9.
84. Fraczek MG, Naseeb S, Delneri D. History of genome editing in yeast. Yeast. 2018;35:361–8.
85. Dicarlo JE, Norville JE, Mali P, Rios X, Aach J, Church GM. Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. Nucleic Acids Res. 2013;41:4336–43.
86. Li M. Engineering of *Saccharomyces cerevisiae* for production of resveratrol and its derivatives. The Novo Nordisk Foundation. 2016;9(1):1–137.

87. Zhang Y, Li SZ, Li J, Pan X, Cahoon RE, Jaworski JG, et al. Using unnatural protein fusions to engineer resveratrol biosynthesis in yeast and mammalian cells. J Am Chem Soc. 2006;128:13030–1.
88. Trantas E, Panopoulos N, Ververidis F. Metabolic engineering of the complete pathway leading to heterologous biosynthesis of various flavonoids and stilbenoids in *Saccharomyces cerevisiae*. Metab Eng. 2009;11:355–66.
89. Beekwilder J, Wolswinkel R, Jonker H, Hall R, de Rie Vos CH, Bovy A. Production of resveratrol in recombinant microorganisms. Appl Environ Microbiol. 2006;72:5670–2.
90. Sydor T, Schaffer S, Boles E. Considerable increase in resveratrol production by recombinant industrial yeast strains with use of rich medium. Appl Environ Microbiol. 2010;76:3361–3.
91. Li M, Borodina I. Application of synthetic biology for production of chemicals in yeast *Saccharomyces cerevisiae*. FEMS Yeast Res. Oxford University Press. 2015;15:1–12.
92. Rodriguez A, Kildegaard KR, Li M, Borodina I, Nielsen J. Establishment of a yeast platform strain for production of p-coumaric acid through metabolic engineering of aromatic amino acid biosynthesis. Metab Eng. 2015;15:1–12.
93. Borja GM, Rodriguez A, Campbell K, Borodina I, Chen Y, Nielsen J. Metabolic engineering, and transcriptomic analysis of *Saccharomyces cerevisiae* producing p-coumaric acid from xylose. Microb Cell Factories. 2019;18(1):191.
94. Sun L, Kwak S, Jin YS. Vitamin A production by engineered *Saccharomyces cerevisiae* from xylose via two-phase in situ extraction. ACS Synth Biol. 2019;8:2131–40.
95. Sauer M, Branduardi P, Valli M, Porro D. Production of L-ascorbic acid by metabolically engineered *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*. Appl Environ Microbiol. 2004;70:6086–91.
96. Levisson M, Patinios C, Hein S, de Groot PA, Daran JM, Hall RD, et al. Engineering de novo anthocyanin production in *Saccharomyces cerevisiae*. Microb Cell Factories. 2018;17(1):103.
97. Eichenberger M, Hansson A, Fischer D, Dürr L, Naesby M. De novo biosynthesis of anthocyanins in *Saccharomyces cerevisiae*. FEMS Yeast Res. 2018;18(4):1–13.
98. Su B, Song D, Zhu H. Metabolic engineering of *Saccharomyces cerevisiae* for enhanced carotenoid production from xylose-glucose mixtures. Front Bioeng Biotechnol. 2020;8:1–11.
99. Kim IK, Roldão A, Siewers V, Nielsen J. A systems-level approach for metabolic engineering of yeast cell factories. FEMS Yeast Res. 2012;12:228–48.
100. Otero JM, Cimini D, Patil KR, Poulsen SG, Olsson L, Nielsen J. Industrial systems biology of *Saccharomyces cerevisiae* enables novel succinic acid cell factory. PLoS One. 2013;8(1):e54144.
101. Brochado AR, Matos C, Møller BL, Hansen J, Mortensen UH, Patil KR. Improved vanillin production in baker's yeast through in silico design. Microb Cell Factories. 2010;9:1–15.
102. Raab AM, Gebhardt G, Bolotina N, Weuster-Botz D, Lang C. Metabolic engineering of *Saccharomyces cerevisiae* for the biotechnological production of succinic acid. Metab Eng. 2010;12:518–25.
103. Ishida N, Saitoh S, Ohnishi T, Tokuhiro K, Nagamori E, Kitamoto K, et al. Metabolic engineering of *Saccharomyces cerevisiae* for efficient production of pure L-(+)-lactic acid. Vol. 795. Appl Biochem Biotechnol. 2006;129:795–807.
104. van Maris AJA, Geertman JMA, Vermeulen A, Groothuizen MK, Winkler AA, Piper MDW, et al. Directed evolution of pyruvate decarboxylase-negative *Saccharomyces cerevisiae*, yielding a C2-independent, glucose-tolerant, and pyruvate-hyperproducing yeast. Appl Environ Microbiol. 2004;70:159–66.
105. Zelle RM, de Hulster E, van Winden WA, de Waard P, Dijkema C, Winkler AA, et al. Malic acid production by *Saccharomyces cerevisiae*: engineering of pyruvate carboxylation, oxaloacetate reduction, and malate export. Appl Environ Microbiol. 2008;74:2766–77.
106. Kogje A, Ghosalkar A. Xylitol production by *Saccharomyces cerevisiae* overexpressing different xylose reductases using non-detoxified hemicellulosic hydrolysate of corncob. 3 Biotech. 2016;6(2):127.

107. Denby CM, Li RA, Vu VT, Costello Z, Lin W, Chan LJG, et al. Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer. Nat Commun. 2018;9(1):965.
108. Heitmann M, Zannini E, Arendt E. Impact of *Saccharomyces cerevisiae* metabolites produced during fermentation on bread quality parameters: a review. Crit Rev Food Sci Nutr. 2018;58:1152–64.
109. Shin SY, Jung SM, Kim MD, Han NS, Seo JH. Production of resveratrol from tyrosine in metabolically engineered *Saccharomyces cerevisiae*. Enzym Microb Technol. 2012;51:211–6.
110. Bae SM, Park YC, Lee TH, Kweon DH, Choi JH, Kim SK, et al. Production of xylitol by recombinant *Saccharomyces cerevisiae* containing xylose reductase gene in repeated fed-batch and cell-recycle fermentations. Enzyme Microbial Technol. 2004;35:545–9.
111. Oh EJ, Ha SJ, Rin Kim S, Lee WH, Galazka JM, Cate JHD, et al. Enhanced xylitol production through simultaneous co-utilization of cellobiose and xylose by engineered *Saccharomyces cerevisiae*. Metab Eng. 2013;15:226–34.
112. Cheng M-H, Sun L, Jin Y-S, Dien B, Singh V. Production of xylose enriched hydrolysate from bioenergy sorghum and its conversion to β-carotene using an engineered *Saccharomyces cerevisiae*. Bioresour Technol. 2020;308:1–18.
113. Hong J, Park SH, Kim S, Kim SW, Hahn JS. Efficient production of lycopene in *Saccharomyces cerevisiae* by enzyme engineering and increasing membrane flexibility and NAPDH production. Appl Microbiol Biotechnol. 2019;103:211–23.
114. Wang M, Wei Y, Ji B, Nielsen J. Advances in metabolic engineering of *Saccharomyces cerevisiae* for Cocoa butter equivalent production. Front Bioeng Biotechnol. Frontiers Media SA. 2020;8:1–8.
115. Almahbashi M, Baldwin G, Byran A, Chang W, Chavez M, Cassab M, et al. Real vegan cheese: Casein production in *Saccharomyces cerevisiae*. iGEM SF-Bay- Area- DIY bio-Championship Posters. Available from: http://2014.igem.org/files/posters/SF_Bay_Area_DIYbio_Championship.pdf
116. Amyris. REALSWEET™ REB M. 2022. Available from: https://amyris.com/ingredient/rebm
117. EVOLVA. Health Ingredients. 2022. Available from: https://evolva.com/business-segment/health-ingredients/
118. Husnik JI, Volschenk H, Bauer J, Colavizza D, Luo Z, van Vuuren HJJ. Metabolic engineering of malolactic wine yeast. Metab Eng. 2006;8:315–23.
119. Brazil. National Technical Commission for biosecurity (CTNBio). Table of microorganisms - commercial use genetically modified micro-organisms and their derivates commercially approved for commercial use in Brazil. 2022. Available from: http://ctnbio.mctic.gov.br/liberacao-comercial/-/document_library_display/SqhWdohU4BvU/view/1687332;jsessionid=E257CCEE4D07707B964D695B4F29B252.columba#/liberacao-comercial/consultar-processo

Chapter 8
Preservation of Natural Food Additives

Eduart Andrés Gutiérrez, Leidy Johanna Gómez, Paula Andrea Méndez, and Laura María Reyes

8.1 Introduction

Characteristics in foods such as color, aroma, crispness, juiciness, among others, are the main attributes that influence the purchase decision by consumers, as well as their microbiological and nutritional stability and quality. During the processing of various food matrices, it is necessary to add different additives in order to improve or maintain properties or strengthen existing ones. According to the Food and Drug Administration (FDA), additives are a variety of natural or synthetic substances added to foods [1, 2]; the latter have been widely used by the food industry, since they are stable and relatively cheap. However, studies have shown adverse reactions derived from the consumption of these synthetic food additives (SFAs), such as cancer, allergic reactions, induction of hyperactivity, and behavioral changes in children [3, 4], which has led to the prohibition of the use of some of these food additives (FAs). The concern of consumers regarding the consumption of SFAs has forced the food industry to find natural food additives (NFAs) as a replacement for SFAs, which are mostly obtained from vegetable and mineral sources. However, in recent years, they have obtained natural ingredients from recycled materials within the circular economy and bioprospecting framework [5, 6].

NFAs can confer nutritional and preservative properties to foods, but they are generally unstable and easily degrade during food processing; among them are bioactive compounds, which are recognized for their efficacy in reducing the risks of diseases such as diabetes, obesity, cancer, chronic leukemia, inflammation, and fighting free radicals [7–10]. Many of these compounds are chemically unstable, primarily when exposed to high temperatures, light, and humidity [10]. Currently,

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to improve the stability of NFAs and other bioactive compounds against adverse conditions, various physical stabilization methods have been used (trapping, encapsulation, adsorption, among others) [11], expanding their range of food applications, mainly in the dairy, meat, fruit, and vegetable industries.

The absorption method, in addition to preserving and stabilizing the properties of different NFAs can also be used for the extraction and concentration of active ingredients from various natural sources. Among the most widely used adsorbents to stabilize, recover and concentrate bioactive compounds, macroporous resins, clay minerals, activated carbon, nanobiomaterials, plant biomass, yeasts, fungi, and some algae have been used [12–14]. Unlike absorption, in trapping, the support material has not been previously developed, since it is obtained in the presence of the active compound, extract, or molecules, which are trapped during the formation of said material (films, hydrogels, and nanocarriers) [15]. Finally, encapsulation allows various active compounds to be trapped within a thin layer of coating material, trapping many biomolecules [16]. The high interest in the food industry in the application of these methods is because, with them, it is possible to preserve the bioactivity and physicochemical stability of natural compounds during the processing or storage of food products, thus protecting it from heat, pH, oxygen, light, humidity, or other extreme conditions. The current chapter aimed to extensively review and analyze the use of adsorption, trapping, and encapsulation as alternatives to stabilize NFAs, as well as to appoint the applications of these new materials as natural additives in the dairy, meat, fruit, and vegetable industries.

8.2 Trapping of Natural Additives

According to the FDA, the additives are a variety of natural or synthetic substances added to the foods. These functional ingredients affect the characteristics of the foods, such as color, flavor, freshness, safety, nutritional value, texture, appearance, and taste. FDA considers the term “natural” to indicate that no synthetic substances were used for food processing. Most of the NFAs are derived from plant and mineral sources [1]. However, natural ingredients have been obtained from recycled materials in the last years, a prominent and sustainable alternative for future research [17]. Table 8.1 summarizes some NFAs, their use, and examples.

Preservation of NFAs allows to maintain the physicochemical properties and functionality of the compounds and molecules that these contain, preventing their degradation during extraction, storage, and application. The physic methods such as adsorption, trapping, micro-and nano-encapsulation are the most used to preserve NFAs, which involve the inter-and intramolecular forces such as electrostatic interactions, hydrogen bond, steric effects, and Van der Waals forces [11] (Fig. 8.1).

Trapping of NFAs is a method where the active compounds, extracts, or molecules are trapped during the formation of a support material such as films, hydrogels, and nanocarriers. Unlike the adsorption method, trapping is a physical method where the material has not been previously developed because it is obtained in the

Table 8.1 Natural food additive types and their uses

Additive type	Example	Application	Reference
Preservative	Ascorbic acid, citric acid, tocopherols (Vitamin E), antioxidants, oils	To avoid biological alterations as fermentation and putrefaction process; so, these maintain freshness	[18–20]
Color	Carmine, anthocyanins, annatto extract, beta-carotene, paprika, turmeric, saffron	To improve the intensity of the color foods or change the color to make them more attractive	[21]
Emulsifier	Pectin Soy lecithin	These substances improve the solubility and dispersion of the insoluble products in a mixture, preventing a separation	[1, 22–24]
Stabilizer	Guar and xanthan gums Pectin	To get and maintain a uniform texture	[1, 22, 23]
Enzyme preparation	Lactase Papain Bromelain Pectinase	Enzymes are used in food processing	[25–28]
Antioxidant	Tannins Phenolic compounds Propolis	To avoid and delay the oxidation process induced by the light and air	[18, 19, 29, 30]

presence of the additive. In this case, the reaction conditions affect the functionality of the NFAs, so it can favor or not their properties [15].

8.2.1 Films

Films with NFAs are a new tendency of active and intelligent packaging, oral degradation films, and sensors [31, 32]. Biodegradable polymers have been used as material support to carry antioxidants, essential oils, stabilizers, emulsifiers, preservatives, and colors. The materials used to form films have shown high potential to carry active ingredients, protect their properties, and improve other materials' properties. Depending on the additive type, the reaction conditions should be studied, such as solvents used, temperature, additive concentration, pH, the solubility of the components, polymer nature, and preparation method (casting, extrusion, compression, spray-drying, laser abrasion, spin coating, plasma irradiation) [33, 34]. Furthermore, the materials must be biocompatible, not toxic, and friendly with the environment for the food industry and biomedical applications. The antioxidants are the most used compounds in films, getting bioactive materials with physicochemical properties improved such as color, adhesivity, homogeneous surface, and stability. Plant extracts with antioxidant activity have shown changes in the coloration of the film, acting as a colorant or affecting the transparency of the films. Furthermore, the concentration of the compounds can modulate the release of them and their effect in the final application; the hydrophobic-hydrophilic nature of the additives influences the trapping efficiency (TE); for these cases, other components have been added to improve the formulations [35]. Gelatin, chitosan, alginate, cellulose,

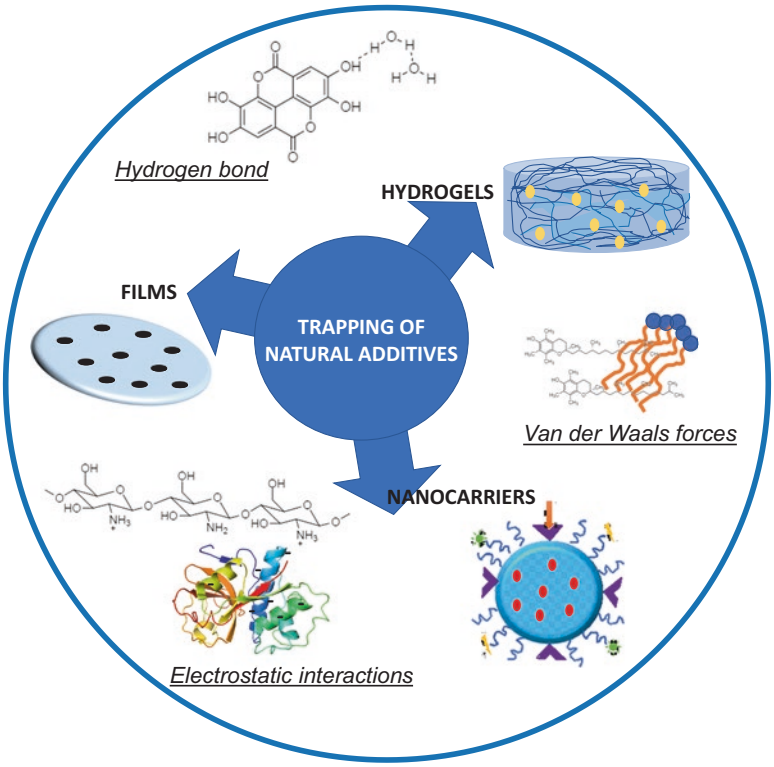

Fig. 8.1 Preservation of natural food additives by trapping and some interactions forces involved in the process

starch, and pectin are examples of natural polymers used to carry bioactive compounds. However, other components such as montmorillonite nanomaterial [32] and silica nanoparticles [36] have been used to reinforce the stability of nanoemulsions and the solubility of additives like essential oils in the polymer matrix. On the other hand, plant extracts (coffee extract with 45 wt.% of polyphenols, cocoa, and cinnamon extracts with 5 wt.% of polyphenols) were used as anti-aging in polylactide (PLA) films. An excess of the extracts was used (0.5; 1; 3; 5 or 10 wt.%), being that the films were obtained by extrusion process. The authors observed that films manufactured with a high extract concentration had changes in surface roughness and adhesive strength, the water vapor permeability was reduced and the oxygen permeability increased, but the active properties of the films were not reported [37]. However, other study about impregnation of cinnamon essential oil (CEO) in films composed of PLA showed an inhibition against *E. coli* and *B. subtilis* by the agar diffusion method, the inhibition was observed in the concentration of CEO between 1–5 wt.%, as the concentration of CEO was increased [38].

Lignin and cellulose nanocrystals have been used as additives to improve film thickness and water resistance. The reactive structure of the lignins due to the

presence of aromatic rings and their diversity in the functional groups provide properties such as dispersing, binding, and plasticization agent [39]. In another study was investigated the films formation from proteins to protect NFAs and using layer-by-layer assembled protein-tannic acid (TA) films (Bovine serum albumin (BSA)-TA and pepsin-TA). In this research was evidenced the stability of the films when immersed in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) for delivery of active food-derived ingredients. To evaluate the stability, the film thickness was evaluated, it was found that pepsin-TA evidenced a major change due to the pepsin, which is an enzyme present in SGF favoring the interaction and the increase of the film thickness. By the contrary, SIF treatment showed a decrease in the thickness due to the digestion process of enzymes such as amylase, lipase, and trypsin. In this work was evaluated the stability of the films and TA was considered an cementing agent; however, the bioactivity was not evaluated after the *in vitro* essays [31]. Trapping of NFAs by the film formation can also be affected by the temperature. The heat-moisture treatment in starch changed some properties such as granule size, gelatinization process, solubility, and crystallinity, which affected the complexation and the interaction forces with the additives [40].

The functionality of the bioactive compounds in the polymer matrix is evidence by means of the preservation of NFAs. In trapping, the polymer matrix can protect the bioactive compound against temperature. For example, films based on polypropylene with active properties were obtained after the blending of phenolic compounds with polymer pellets, 0.2 wt.% of Irganox 1010 and tomato extract 2 wt.% were used as antioxidant additives. In this study films were manufactured by pressing the blended polymer chips at 200 °C and 50 MPa, after the blending bioactive additives with polymer pellet at 180 °C, confirming the thermal stabilization of active compounds during the film's processing [41]. Other active ingredients as peptides and proteins as food preservatives have been used in films getting antimicrobial and antioxidant activity after the interaction with the polymer and the forming of the film [42]. The compatibility between the NFAs and the materials is an important characteristic, but the stability of the NFAs in the material and after the different treatments must be analyzed. The effect of the temperature on the adsorption process of anthocyanins on the Laponite® (Lap), a commercial colloidal clay, evidenced a stability of this biohybrid. Anthocyanins were extracted from Jambolan fruits and the adsorption was 15.98 mg of cyanidin-3-glucoside equivalent/g of Lap; this process was independent of the temperature in the range of 5–40 °C. The activity of the functional compounds after the desorption process evidenced a better antioxidant activity, the anthocyanins adsorbed in the Lap and the crude extract inhibited 58.14% and 70% the DPPH radical, respectively [43]; possibly the inorganic nature of the Lap contributes to the stability of this pigments improving their activity [44].

8.2.2 Hydrogels

Hydrogels are three-dimensional polymeric networks that can absorb and retain water. These materials have hydrophilic nature due to the presence of functional groups such as carboxyl (–COOH), hydroxyl (–OH), and amino ($–NH_2$) groups. Hydrogels have the swelling and wetting capacity; however, they cannot be dissolved in water. Hydrogels have been used as thickeners and stabilizers, edible films, confectionery, yogurt, and gel-type products in the food industry. Unfortunately, these materials have a low loading capacity of bioactive compounds and the mechanical strength is too weak. Most hydrogels are produced using polysaccharides and proteins obtained from natural sources, widely known by their biodegradability and biocompatibility. Since food safety must be guaranteed, the physical methods to prepare hydrogels are the most used, physical cross-linking, heating-cooling, ionic interaction, complex coacervation, hydrogen bonding, maturation, and enzymatic-induced crosslinking. Although these methods have limitations in the loading capacity of the bioactive substances and gel stability, these can be reversible by not using cross-linking agents, achieving weak interactions [45–48]. Studies have been carried out to improve the stability and mechanical properties of hydrogels, particularly, variables such as pH, polymer concentration, and temperature have been investigated to modulate the physical interactions (ionic, H-bonding, hydrophobic) between polymers or substances of the formulation. Recent works have shown that chitosan forms gel under neutral and basic conditions, and sodium alginate experiments gelation in a narrow pH range; however, the interaction between chitosan-sodium alginate forms robust composite hydrogels over a wide pH range getting significant changes in viscoelastic properties. In addition, these physical cross-linking hydrogels evidenced the trapping of citral with a efficiency of 77.5% with chitosan at pH 7, an natural flavoring compound that is unstable in acidic aqueous solutions; it explains the importance of the variables in the preparation method, in this case the pH of the ionic polymers chitosan and alginate, and the stability of the NFAs [49]. On the other hand, new strategies have been explored to improve the trapping of NFAs, hydrogel beads containing liposomes with incorporated bioactive compounds, α-linolenic acid (0.804 ± 0.012%) and quercetin (0.158 ± 0.003%) were obtained. Liposomes were added and the hydrogel was formed with chitosan by injection-gelation method; the content of the α-linolenic acid (0.986 ± 0.037%) and quercetin (0.194 ± 0.010%) increased; due to the dynamic equilibrium of the ion crosslinking the hydrophilic phase of the liposome was stabilized and the hydrophobic phase trapped the bioactive compounds. Hence, the combination of them integrates advantages [50].

Finally, NFAs can be used to form hydrogels, in this way, phytic acid (PA) 1 M, an organic acid present in vegetables can be used as physical crosslinker with the polycation poly(trimethylamino)ethylmethacrylate chloride (pTMAEMA), their interactions as multivalent ion and hydrogen bonding formed the hydrogel, getting to improve rheological properties and antibacterial capability which was

proportional to the concentration of PA, so, the annular radius increased in gram-negative *E. coli* and gram-positive *S. epidermidis* [51].

8.2.3 Nanocarriers

Nanotechnology is related to the manipulation of structures at the nanoscale (1–100 nm). In recent years, several research studies have focused to explore the use of nanotechnology in the food industry, hence, the use of nanoadditives, nanoceuticals, and the nanoencapsulation are examples of nanotechnological applications in the food sector [52]. Furthermore, the food packaging industry has explored the use of nanomaterials, in this way, different food packaging can be manufactured containing antimicrobials agents or receptors, both at nanometric scale and used to reduce the microbial growth or detect pathogens in foods, respectively [53]. Nanocarriers are obtained from organic or inorganic materials, natural or synthetic polymers, hydrophilic or hydrophobic macromolecules [54]. These nanostructures can be used as carrier agents with controlled delivery, protecting the antioxidant and antimicrobial properties of NFAs [53, 55, 56]. Different nanocarriers have been explored in the last years, including liposomes, inorganic nanoparticles, polymeric nanoparticles, solid lipid nanoparticles, dendrimers, quantum dots, micelles, nanofibers, nanogels, nanocomposites, and nanocages. Chitosan nanostructures have been studied as systems to encapsulate vitamin C (70%) by the method of ionic gelation, curcumin (90%) by coacervation and enzymes by electrospinning (Norep). Ferritin nanocage was studied in the encapsulation of epigallocatechin gallate (EGCG) and curcumin obtaining a loading efficiency less than 2% [52, 56, 57].

According to the nature of additive (ionic, hydrophilic, hydrophobic), the nanocarrier material and the preparation method are selected because the trapping efficiency depends on the physical interactions between nanocarrier material and bioactive compounds. So, the polycation chitosan favors the ionic gelation with tripolyphosphate (TPP) to encapsulate hydrophilic molecules as vitamin C getting an encapsulation efficiency (EF) of 95% [52]; but compounds with lower hydrophilicity as Resveratrol has been encapsulated in sodium carboxymethyl cellulose by emulsification getting EF of 58%. Curcumin, a polyphenol with antioxidant properties, has shown a broad spectrum of biological functions due to their properties anti-inflammatory, anti-microbial, anti-diabetic, and anti-cancer. However, this molecule is unstable chemically and has low bioavailability, limiting its use. Different strategies have been studied to trap this natural additive using nanoliposomes, lipid solid nanoparticles, polymeric nanoparticles, and dendrimers. For these cases, trapping efficiency (TE) depended on the type of molecule used to form the nanocarrier and the preparation method. Nanoliposomes prepared by thin film hydration using soybean phospholipids showed a higher TE (82.3%) for curcumin than soy lecithin (34.7–70%) and cholesterol (57%); the amphiphilic behavior of the phospholipids incorporates between the liposomal layer the lipophilic compounds and the hydrophilic segment of the phospholipid stabilize the system getting

a higher TE. Poly (lactide-co-glycolide) (PLGA) nanoparticles showed a high entrapment efficiency of curcumin by using nanoprecipitation and emulsification solvent evaporation-like preparation methods, 90% and 90.8%, respectively. The hydrophobic nature of the polymer and the molecular size of the material favors the interactions with Curcumin and the high TE [55, 58].

Nanoparticles can protect NFAs from physiological conditions, so these nanostructures are an alternative to improve the bioavailability of bioactive ingredients in the gastric medium. For example, alginate beads encapsulated jabuticaba peel extract with EF of 89.6%; the release profile in simulated gastric media (pH 1.2) showed that the 43.1% of the anthocyanins were released after 240 min and their color remained, which suggest that alginate beads protected the anthocyanins present in the extract [59]. On the other hand, nanocarriers like liposomes, micelles, and nanoemulsions have shown potential application in preservation, reducing the impact of taste and enhancing the antimicrobial activity of some bioactive compounds like propolis, a natural preservative with antiseptic, antimicrobial, and antioxidant properties [18]. For example, the micelles have been used to improve the solubility of the bioactive compounds; curcumin solubility was improved with their trapping in beta-casein micelles (B-CN), the change in fluorescence intensity (35 a.u at 50 μmol.L^{-1} of B-CN) and the absorption spectra of curcumin in the presence of B-CN, showed a shift toward lower wavelengths (blue shift), which evidences changes in the polar environment [60, 61].

The bioactive molecules have functional groups of hydrophilic (hydroxyl, carboxyl, amino, and carbonyl) and hydrophobic (aromatics, alkenes, alkynes, and cycloalkanes) nature, favoring the inter-and intramolecular forces with the entrapment material. Inorganic nanoparticles have been used in recent years due to their different carrier matrix and hydrophobic nature. Silica nanoparticles with size close to 200 nm showed high potential to load curcumin (70%), but volatile thymol evidenced a lower loading efficiency (23%). In addition, microparticles (1500 nm) showed higher loading efficiency due to the molecules were adsorbed onto the outer surface, 100% for the curcumin and 33% for the thymol. The porosity of the inorganic material influenced the encapsulation efficiency; lower surface area with accessible pore structure (1–2.5 nm) favored the encapsulation, however the release kinetic was not favored in microparticles due to the burst effect caused by the adsorption of the molecules in the surface, the 80% was released in 24 h in culture media, and the 50% in 65 h in food simulated media [62].

8.3 Encapsulation of Bioactive Compounds and Food Additives

Encapsulation is defined as a simple process during which a core material (active food ingredients, bioactive compound, enzymes, or other compounds) is entrapped inside a thin layer of coating material (shell); this is an interesting technique because

they entrap a considerable number of biomolecules [63]. Encapsulation term is originally from biotechnology and pharmaceutical field, where it was used to protect sensitive drug and vaccine delivery. However, in recent years, the application of encapsulation technique in the food industry has been grown much rapidly, mainly for the encapsulation of NFAs such as colorants, preservatives, flavors, vitamins, minerals, and bioactive compounds [64, 65]. Nowadays, encapsulation is used to preserve the physicochemical properties and bioactivity of NFAs during food processing and storage, thus protecting from heat, pH, oxygen, light, moisture, or other extreme conditions. Furthermore, encapsulation can be utilized to restrict undesirable interactions between the core material and other food ingredients [16]. In addition, encapsulating bioactive compounds can mask the core's undesirable taste, color, or odor so that sensory attributes are not compromised, increasing the consumer acceptance. It can be used to design controlled release formulations to achieve targeted and sustained delivery of bioactive compounds [63]. The encapsulation technique can be categorized according to the arrangement of the core into: (i) micro/nanocapsules, which is a vesicular system where the active substance is confined and surrounded by wall material; and (ii) micro/nanospheres that are a matrix system, in which the active substance is uniformly dispersed in the wall material [66, 67]. On the other hand, based on the size, the encapsulation technique can be broadly categorized into microencapsulation (2–800 μm) and nanoencapsulation (1–1000 nm); both methods have their advantages and disadvantages, but the nanoencapsulation technique is considered to be the better approach because can enhance the bioavailability, controlled release, and enable precision targeting of the active compounds to a greater extent than microencapsulation [68].

Many factors can influence encapsulation efficiency, mainly core material, carrier/matrix, and encapsulation technique. The core material should be considered numerous properties, including concentration, solubility, size, diffusivity, and possible interactions with other components [69]. On the other hand, according to the type of carrier/matrix; some properties such as encapsulant materials, composition, and rheology of phases, the physical state of phases, original size, porosity, shape, and structure, could affect encapsulation efficiency and release rate of the compounds [70]. Depending on the encapsulation technique, it is possible to use different types of wall material, including polysaccharides, proteins, or lipids. For food application, materials used in the encapsulation systems should satisfy some conditions from the health and safety point of view, such as being approved as "generally recognized as safe" (GRAS) substances being biodegradable rather than natural origin. Moreover, the chosen carrier should be able to maintain physicochemical properties and preserve the potency of the encapsulated compound and mask any unpleasant appearance or flavor of the compound but without altering the organoleptic properties the food product, [71]. Some of the most popular wall materials used for micro nanoencapsulation application in food are: arabic gum, maltodextrin inulin, modified starches, alginate, casein, isolate and concentrate whey gelatin or soy protein, vegetable oil, and hydrogenated fats [72, 73]. Other factors that can affect the successfulness of the encapsulation process are the emulsifier and surfactant type and concentration, the pH of the solution, and environmental conditions

such as temperature and the presence of ions [70]. The choice of the encapsulation technique depends on the physicochemical properties of core and wall materials, carrier matrix stability, physical properties required in encapsulates such as size and shape and cost material [74], some characteristics of the different encapsulation techniques are described below.

8.3.1 Encapsulation Techniques

There are several techniques, so the selection of the method is based on the budget, costs, core, the desired size of the capsules, and the release mechanisms [67]. Broadly the methods are divided into three types: (i) chemical methods that include polymerization and cross-linking; (ii) physicochemical methods including ionic gelation, coacervation, supercritical assisted encapsulation, emulsion, and molecular inclusion complexes; and (iii) physicomechanical processes such as spray drying, freeze-drying, solvent evaporation and fluid bed coating [16, 75]. Some of the main ones used in the food industry are discussed.

8.3.1.1 Simple and Complex Coacervation

Coacervation is considered the original encapsulation method. It is defined as separating aqueous colloidal solution into two liquid phases. One is rich in a polymer (coacervate phase), and another is poor in a polymer called equilibrium solution [75]. Coacervation techniques can be divided in aqueous phase, which can only be used to encapsulate water-insoluble materials, or organic phase which allows the encapsulation of water-soluble material [76]. On the other hand, two methods for coacervation, namely simple and complex processes, differ by how the phase separation is carried out [70]. Complex coacervation has advantages over simple coacervation, because it can produce microcapsules with smaller particle sizes and gives an unusually higher payload of up to 90% for single-core and 60% for multicore than other microencapsulation processes [75, 77].

8.3.1.2 Ionic Gelation

This is an easy technique based on the capacity to cross-link polyelectrolytes in the presence of multivalent ions such as Ba^{2+}, Ca^{2+}, and Al^{3+}, being used to encapsulate hidrophylic/emulsified or hydrophobic compounds [76]. Ionic gelation can be carried out by extrusion or emulsification/gelation. It is necessary to use coating materials, which can be gums, carbohydrates, celluloses, lipids, proteins, and inorganic materials, and a crosslinking solution of multivalent ions, usually calcium ions [75]. Encapsulation by gelation technique could be performed either externally or internally. External gelation is the most common and easy for both types of compounds,

soluble and insoluble. It produces heterogeneous gels and provides larger capsule sizes (>2000 μm) and encapsulation efficiency ranges from 65% to 95% approximately [78, 79]. On the other hand, a uniform capsule size and smooth surface are obtained when internal gelation is induced, making caking less agglutination and, therefore, less cracking or pores [80]. Alginate is the most common wall material used in the ionic gelation technique since its non-toxic profile, solubility, and biocompatibility. Further, it shows high toughness, has considerable effects on the mechanical stability of beads [70] and can encapsulate macromolecular and low molecular weight agents [75].

8.3.1.3 Spray Drying

This process involves core particles dispersion in a carrier material (polymer solution), followed by the atomization of the mixture in a hot chamber, causing uniform and rapid solvent evaporation by direct contact [75]. Various natural polymers can be used as wall materials in spray drying technique, especially polysaccharide-based encapsulation materials such as gums, starches fructooligosaccharides, alginates, and their derivatives [81]. Spray drying has some advantages over other encapsulation techniques such as low-cost production, fast processing, and high productivity, so it is a technique widely used in the food industry to extend the shelf life of a wide variety of components, including flavors, colors, vitamins, minerals, fats, and oils [82]. However, spray drying also has some disadvantages, such as loss of active compound due to high temperatures, presence of core material on the surface, and limited availability of core materials [67].

8.3.1.4 Freeze-Drying

This technique includes three principal stages, firstly freezing followed by a primary drying where ice or other frozen solvents are removed from the material by sublimation, and a secondary drying where bounded water is removed by desorption [75]. The third stage is a treatment to prevent rehydration of the material [67]. Before the freeze-drying process, the core material must be dissolved, dispersed or emulsified into the wall material [72]. The main advantage of freeze-drying is to keep a low temperature in the product during the process, whence is a suitable technique that could maintain the characteristics of many heat-sensitive products such as anthocyanins, oils, probiotics, enzymes, among others [75]. However, this technique exhibits high energy use, long processing time, and high production costs [67].

8.3.1.5 Interfacial Polymerization

In this process, the polymerization of a hydrophilic and lipophilic monomer occurs at the interface of an oil-in-water emulsion, forming a membrane, which will give rise to the wall of the microcapsules [75]. This process involves three steps: (i) dispersion of an aqueous solution of a water-soluble reactant in an organic phase to produce a water-in-oil emulsion; (ii) formation of a polymeric membrane on the surface of the water droplets, initiated by the addition of an oil-soluble complex to the previous emulsion; and (iii) separation of the microcapsules from the organic phase and their transfer in water to give an aqueous suspension [66]. Interfacial polymerization has potential benefits, including high loading of the active compound, low cost, easy-to-scale, simplifying, and process reliability. The method does not need catalysts and can be performed at low temperatures, allowing to control the capsule size and membrane thickness. However, some factors limit the application of this technique, mainly because it presents a challenge for controlling large oil-water interfaces via interfacial polymerization and to assure yield and membrane quality [75].

8.3.2 Nanoencapsulation Approaches

Nanoencapsulation is a promising new technology and has some advantages over microencapsulation in targeted site-specific delivery of encapsulated compounds and improved controlled release [83]. Moreover, the nanoscale size leads to greater efficient absorption through cells by prolonging gastrointestinal retention time due to improved bioadhesiveness in the mucus covering the intestinal epithelium, providing high solubility and preserving included compounds [68]. On the other hand, particles at the nanoscale have a high surface-to-volume ratio, increasing their reactivity with modifications in viscoelastic properties [16]. It has been effectively shown that nanoencapsulation can preserve compounds from degradation in food processing, providing enhanced stability, retention of volatile ingredients, and protection against oxidation and other environmental factors [84]. Furthermore, nanoparticles create a more stable system as they have less tendency for particle aggregation or gravitational separation in foods. The nanoencapsulation techniques are more complex than microencapsulation techniques, mainly due to the difficulty in attaining the complex capsule and core material morphology and the demands of controlling release rate in nanoencapsulated materials [67]. Several nanoencapsulation methods include nanoparticles, nanodroplets, nanoemulsions, and nanohydrogels. The selection depends on different parameters, including chemical and physical characteristics of the core and shell materials, particle size, and the required rate of release and delivery of encapsulated compounds [74].

Some techniques developed and used for microencapsulation purposes include emulsification–solvent evaporation, inclusion complexation, coacervation, ionic gelation, anti-solvent precipitation, coacervation, spray drying, and supercritical

fluid techniques, can be considered to be nanoencapsulation techniques because they can produce capsules in the nanometer range [85]. Moreover, emerging and promising approaches to produce nanocapsules or nanofibers like electro-spraying and electro-spinning have been of interest in recent years due to the high efficiency in encapsulation, facility, and cost-effective [67, 70]. On the other hand, the nanoencapsulation techniques can be divided into top-down, which involves the application of the precise tool that allows size reduction and shaping the structure of the nanomaterial, or bottom-up approaches, in which materials are constructed by self-assembly and self-organization of molecules. The bottom-up approach is influenced by many factors such as pH, temperature, concentration, and ionic strength, and it includes supercritical fluid techniques, inclusion complexation, coacervation, and nanoprecipitation techniques. In contrast, the top-down approach uses emulsification and emulsification-solvent evaporation techniques [68].

8.3.3 Perspective on Challenges and Future Trends

A high number of research regarding new technologies and advances for obtaining desired characteristics of micro or nano-capsules, e.g., the combination of two encapsulation techniques such as emulsification with spray drying or electrospinning, has recently been studied, offering combined benefits [67]. Similarly, the combination of two or more carrier materials such as polysaccharides with protein or lipid, or a combination of polysaccharides, has recently received attention because they have shown advantageous effects on compounds retention and release characteristics [83].

Another concept recently introduced in food industries is the co-encapsulation of two or more active functional ingredients in a single matrix. This application offers multiple benefits and synergies with improved bioactivity and functionality than Campo's single component [63]. Co-encapsulation concept has been widely popularized for pharmaceutical products. It has vast possibilities as a potential single micro delivery vehicle for probiotic strains and prebiotics, essential oil, vitamins, antimicrobial compounds, among others. Nevertheless, there is very limited research regarding the formulation of functional food products based on co-encapsulation, so it is a topic that still needs to be investigated [73]. Different methods such as emulsification, spray drying, freeze-drying, coacervation process, or electrospraying can be used for co-encapsulation of bioactive compounds and food ingredients. However, someone could present drawbacks; for example, the extrusion process leads to a lower payload, and the freeze-drying method is costly [86]. On the other hand, the co-encapsulation products have shown to be more hygroscopic due to the readily release of core ingredients because of their amorphous nature [87].

Growing consumer concern for the environment has resulted in greater food by-products use. In this context, the study of materials from food by-products as a "green" wall material to encapsulate bioactive and food ingredients has increased the interest as new and sustainable alternatives [88]. For example, Marson et al. [89]

evaluated yeast cells from brewery waste as encapsulating materials. This material presents some advantages, such as low cost, less than 20 μm, and high amounts of proteins. The use of food by-products as carrier materials for encapsulation represents an opportunity since it has low cost, multiple technological properties, and availability. Moreover, proteins with emulsifier properties can be extracted from food by-products, so the highest advances are associated with the emulsion's production, which could replace synthetic emulsifiers [65].

Some future challenges regarding encapsulation techniques include the development of solvent-free and environmentally friendly methods, developing economically feasible and easily scaleup-able methods for helping their commercialization, and validation of data obtained from in vitro studies with in vivo experiments should be addressed in future research [90]. On the other hand, there is limited information on the digestion of nano/microencapsulated compounds. In vitro and vivo trials could be important future research direction to explain controlled and targeted site-specific delivery of different food ingredients and bioactive compounds encapsulated and the degree of cellular uptake of compounds in the encapsulated form [77].

8.4 Adsorption of Natural Additives

Adsorption is a surface phenomenon in which a molecule (known as adsorbate) is adhered to a generally solid surface (adsorbent). Since it is a surface phenomenon, it is assumed that there is no diffusion inside the adsorbent [91, 92]. An essential condition for adsorption is the surface of the adsorbent. An adsorbent with more surface has more capacity to adsorb a more significant amount of absorbate [93, 94]. For this reason, the best adsorbents are porous substances, or more generally those with the largest surface area per unit volume (activated carbon, clays, synthetic resins and, biopolymers) [3–6]. The reverse process of the adsorption phenomenon is the desorption process. In other words, the release of adsorbed molecules. Like the adsorption process, desorption plays an essential role in many technological applications for instance, in foods [14, 95, 96], cosmetics [74, 97, 98] and pharmaceuticals [10, 27, 74].

The adsorption process occurs mainly through physical (physisorption) or chemical adsorption (chemisorption). In the first, the adsorbent adheres to the surface through weak electrostatic forces (Van Der Waals interactions, dipole-dipole interactions), forming even multilayers. In chemisorption, the formation of covalent bonds keeps the adsorbate molecules strongly attached to the surface of the adsorbent. Only a monolayer of adsorbate can be chemisorbed to the adsorbent surface [30, 91, 99, 100]. For the adsorption process to occur, low activation energy is required; that is, equilibrium can be reached quickly if the physicochemical conditions of the environment do not change significantly over time. For this reason, adsorption is usually described by a time-independent relationship between the amount of adsorbate attached to the adsorbent and the amount in the environment. Such relationships are called isotherms, which means that their validity is limited to the case of a constant temperature [91, 101–103].

Table 8.2 Adsorption isotherm models most used to study the characteristics of adsorbate-adsorbent systems

Model	Equation	Application	Reference
Langmuir	$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$	Adsorption and desorption of flavonoids of the lemon peel by means of graphene oxide.	[104, 105]
		Amberlite XAD-7HP resin for adsorption to separate and concentrate procyanidins from cranberry pomace.	[106]
		Anthocyanin adsorption on montmorillonite clays	[107, 108]
Freundlich	$q_e = K_F (C_e)^{1/n}$	Brown algae and fungus as biosorbents of copper and lead ions	[109]
		Graphenic-biopolymeric composites for adsorption of crocin from saffron extract	[110]
		Adsorption of gallic acid (GA) and propyl gallate (pG) on activated carbon (Ac)	[111]
BET	$q_e = \frac{q_m K_{BET} X}{(1-X)\left[1+(K_{BET}-1)X\right]}$	Phenol adsorption from aqueous solutions using Pinus pinaster bark.	[112]
		Organically activated bentonite and a humic acid polymer in mycotoxin adsorption.	[113]
		Adsorption of maltodextrin or gum arabic by chicken protein hydrolysate powder	[114]

q_e (mg $*$ g^{-1}): Solute adsorbed per unit weight of adsorbent at equilibrium
C_e (mg $*$ L^{-1}): Equilibrium concentration of the solute in the bulk solution
q_m (mg $*$ g^{-1}): Maximum adsorption capacity
K_F (mg $*$ g^{-1})(mg $*$ L^{-1})$^{-1/n}$: Constant indicative of the relative adsorption capacity of the adsorbent
K_F (mg $*$ g^{-1}): Constant indicative of the relative adsorption capacity of the adsorbent
n: Indicates the intensity of the adsorption
K_{BET}: Equilibrium constant
$X = C_e/C^*$
C^* (mg $*$ L^{-1}): Saturation solubility

Several isotherms' models are used to describe the behavior of the adsorbate-adsorbent interaction. The most used are the Langmuir isotherm [104], the Freundlich isotherm [91, 102] and the Brunauer, Emmett and Teller (BET) isotherm [102]. Table 8.2 summarizes the main characteristics of the most common models employed in the adsorption process study, highlighting bioactive compounds stabilization, extraction, and concentration. Like many other physicochemical phenomena, adsorption is influenced by system conditions, particularly by pH, temperature, and the redox properties of the involved species; due to these limitations, it is of interest to know the optimal conditions in which the system reaches the maximum adsorption capacity.

8.4.1 Adsorption in the Food Industry

Properties such as color, aroma, and antioxidant capacity are the main attributes that influence the decision by consumers in the choice and acceptance of foods. Sometimes when processing foods, it is necessary to add different additives to improve new properties or strengthen existing ones [1, 115]. The food industry has widely used SFAs as they are stable and relatively inexpensive [21]. However, studies have shown adverse reactions derived from the consumption of these FAs, such as cancer, allergic reactions, induction of hyperactivity, and behavioral changes, that have led to prohibiting some of these SFAs in foods. Consumer concerns regarding the consumption of SFAs have forced the food industry to find natural sources to replace them [116].

NFAs give foods useful health, nutrition, and preservation properties, but they are generally unstable and quickly degrade during food processing. Many of these compounds are chemically unstable, primarily when exposed to high temperatures, light, and moisture. Currently, to improve the stability of NFAs and other bioactive compounds against adverse conditions, various physicochemical techniques have been used such as trapping [117], encapsulation [69] and, adsorption [107], expanding their range of food applications.

Adsorption processes have shown great versatility in preserving and stabilizing the properties of NFAs. Also, this technology is very versatile as it can also be used to extract and concentrate active ingredients from many natural sources and their by-products, increasing the natural additive bioavailability [10].

The adsorbents used in the adsorption process can be divided into two main groups: natural and engineered or processed adsorbents [118]. Among the most widely used adsorbents to stabilize, recover and concentrate bioactive compounds, the studies have demonstrated that macroporous resins with different chemical and physical properties could be a simple, environmentally sustainable, and efficient approach for enriching polyphenols compounds. The adsorption/desorption behaviors, kinetics, and thermodynamics for polyphenols compounds' adsorption are investigated using different macroporous resins systems. The results indicated that macroporous resins could be utilized for the large-scale production of polyphenols compounds from fruits, vegetables, and their by-products. Such highly concentrated polyphenols compounds production might expand their application as a biologically active agent for the food industry and pharmacy [13, 106, 119]. Also, zeolites which are intrinsically microporous aluminosilicates of molecular dimensions that allow the adsorbate molecules to infiltrate into these pores, have been exploited in adsorption technologies. The process of adsorption and desorption of molecules in zeolites are based on differences in molecular size, shape, and other properties such as polarity [11, 118, 120, 121]. The application of natural clays as adsorbents of bioactive compounds has been studied using anthocyanins and other natural dyes for a relatively long time [108, 122, 123]. High adsorptive capacity properties of clays such as montmorillonite and saponite are due to their negative structural charges. The negative charge makes them potential adsorbents for positively charged species [107, 124, 125]. Many other materials such as activated carbon [111],

nanomaterials [92], plant biomass [126], yeasts, fungi, and some algae [109, 127] have been commonly used to adsorb mainly natural dyes, an antioxidant compounds, and heavy metal cations. The interest in the possible enhancing effects of NFAs on human health has motivated the evaluation of various aspects of the adsorption process, such as adsorbent structure, bioactive compound/adsorbent interaction, stability, and antioxidant capacity adsorbate-adsorbent hybrid composite.

The design of adsorption processes has played an essential role in developing various technologies. It has economically boosted different productive sectors such as food, petrochemical, pharmaceutical, agricultural, to mention a few. The functionality and simplicity of this technology encourage its use in novel applications, especially in the food sector, in which it has been exploited as mentioned above in the stabilization of NFAs, improvement of extraction processes and concentration of bioactive compounds, development of controlled release systems, design and manufacture of nutraceuticals, smart packaging materials, and among others [10, 128, 129].

Anthocyanins, water-soluble pigments widely distributed in the plant kingdom, change their hue depending on the pH; their stability depends on temperature, light absorption, and the presence of oxygen. Betalains are red, yellow, pink, or orange pigments derived mainly from beets. These show limited solubility in water, and their stability depends mainly on exposure to light and changes in pH. Curcumin, also called diferuloylmethane, is the main natural polyphenol found in the rhizome of curcuma longa whose stability depends on exposure to light and changes in pH. Carotenoids are yellow, orange, and red pigments synthesized by plants. In diets, the most common carotenoids are α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene [10, 97, 113]. Phenolic and non-phenolic compounds are suitable as natural additives for food, cosmetics, and dietary supplements due to the demand for healthier products and their antioxidant properties [103]. As mentioned in this section, bioactive compounds have been extracted and separated from the solution by adsorption operation onto the surface of different adsorbents. The adsorption process for extraction of bioactive from fruits, vegetables, and their by-products usually are adjusted by Langmuir, BET, and Freundlich models. All models have been shown an excellent fitting ($R^2 \geq 0.98$), allowing us to know optimal conditions for the stabilization, purification, and recovery of bioactive compounds. These parameters are essential to maintaining the stability of the extracted compound, ensuring that the health-beneficial properties of these substances are not altered.

8.5 Food Applications

Current markets increasingly demand the use of natural products as substitutes for SFAs in foods, which have traditionally been used for their characteristics as antimicrobial agents, antioxidants, anti-browning, as well as to improve the nutritional

and sensory properties of foods [19, 130]. However, several studies show that excessive consumption of SFAs are related to respiratory, neurological, gastrointestinal and dermatological adverse reactions [19, 131, 132]. To enhance the use and application of GRAS additives [2] with biological activity, totally or partially substituting SFAs, techniques such as encapsulation, entrapment, adsorption, among others, have been studied [19, 88, 133]. Other studies point to the potential use of these substances to develop edible or biodegradable films and coatings that allow extending the shelf life of food [134–137].

8.5.1 Dairy Products

Some studies show the importance of incorporating plant-based or fruit-based additives to fortify dairy products, due to their limited content of bioactive compounds [19, 138, 139]. Some authors have carried out studies for the fortification of yogurts with different antioxidants, *Matricaria recutita* L. (chamomile) and *Foeniculum vulgare* Mill. (fennel) rich in phenolic compounds such as quercetin-3-O-glucoside and 5-O-caffeolylquinic acid, or di-caffeoyl-2,7-anhydro-3-deoxy-2--octulopyranosonic acid and luteolin-O-glucuronide, respectively [19], as well as their efficient use as preservatives in cottage cheese [140, 141]. On the other hand, have been used bioactive dairy peptides and a mixture of lotus seed and lily bulb powder in goat yogurt resulted in a fermentation time reduction, water holding capacity improvement, and inhibition of post-acidification of goat yogurt during storage. The addition of both supplements was found to be useful in making up for the two defects of goat yogurt, prolonged fermentation, and soft curds. Therefore, bioactive dairy peptides and lotus seeds/lily bulb powder are recommended to be added to goat yogurt as nutritional supplements [142].

Other studies have shown the antimicrobial activity of bioactive compounds for the preservation of dairy products, specifically cheese. The added moringa oleifera extract to cream cheese at different ratios 2.00, 3.00, and 4.00 g/100 g extend its shelf life up to 4 weeks and increase the probiotic counts, total phenol content, and antioxidant activity of finale products [143]. In cottage cheese, it was added decoctions of *Foeniculum vulgare* Mill. (fennel) and the antioxidant and antimicrobial potential was evaluated. The incorporation of fennel-based ingredients did not alter significantly the nutritional characteristics of control cottage cheese (without fennel-based ingredients) but avoided the increase in yellowness (after 7 days of storage) and the decrease in lactose content (after 14 days of storage) observed in control samples [140].

In other products such as butter and ice cream, the effect on the nutritional, physicochemical, and microbiological quality of the incorporation of essential oils, plant extracts, peptides, among others, has also been evaluated. Ginger rhizomes in pulp, juice, candy, and powder were used and added to the ice cream mix during the freezing step. The addition of different forms of ginger decreased the fat and protein content and increased the ash and fiber content of the resulting ice cream. Antioxidant activity and total phenols were significantly increased by adding ginger in different

forms. In addition, ice cream overrun was reduced and melting resistance increased with the addition of ginger preparations [144].

8.5.2 Meat Products

Meat and meat products are rich sources of nutrients. Still, deterioration problems can occur during the storage and distribution period mainly due to microorganisms and loss of flavor and aroma due to molds and yeasts. That is why the incorporation of natural additives has been proposed as an alternative to prevent or reduce the growth of pathogenic and deteriorating microorganisms in this kind of food [132, 145–148]. To improve the stability of active compounds such as essential oils, techniques such as encapsulation have been used [145, 149]. This strategy protects the oil by one or more layers of a coating agent, avoiding their interaction with the food components and increasing their bioactivity [150]. Moreover, a controlled release of active compounds occurs and masks the intense odors associated with essential oils [151]. This strategy would reduce essential oil in meat products, resulting in a safe and good quality [152].

Adding essential oils such as coriander in low concentrations (0.075–0.150 μL/g) has a significant effect on the conservation of cooked pork sausage, presenting a reduction in the residual nitrite concentration and limiting microbial growth [147]. Another study shows the effect of cinnamon oil nanoemulsion for its antimicrobial and antioxidant efficacy to preserve quality and extend the shelf-life of *Asian seabass* (Lates calcarifer) fillet during chilled storage. During storage, cinnamon oil nanoemulsion was effective in inhibiting the growth of bacteria and oxidation. The shelf-life of the fillets was 2–4 days for the control and at least 6–8 days for the cinnamon oil nanoemulsion-treated samples [153]. Other studies with essential oils, fruits, and vegetable extract are presented in Table 8.3.

Table 8.3 Active compounds used for the preservation of meat and meat products

Natural additive	Property	Food application	Reference
Cinnamon essential oil and grape seed extract	Antimicrobial (*Clostridium perfringens*) and antioxidant activity	Lyoner-type sausages	[154]
Clove essential oil and grape seed extract	Antimicrobial and antioxidant activity	Raw Buffalo Patty	[155]
Clove and cinnamon essential oil	Antimicrobial activity (Listeria monocytogenes)	Ground beef	[156]
Thyme essential oi	Antimicrobial and antioxidant activity	Beef burgers	[157]
Cuminum Cyminum L. aqueous extract	Antimicrobial and antioxidant activity	Sardine fish	[158]
Thyme, Cannelle and Oregano essential oil	Antimicrobial activity (*S. enterica, S aureus,* and *E. coli*)	Chicken	[159]
Clove, sage and kiwifruit peel extracts	Antimicrobial and antioxidant activity	Fish fingers	[160]

8.5.3 Edible and Biodegradable Films and Coatings

The interest of the food packaging sector in bioactive compounds is based on their lack of toxicity, being designated as GRAS compounds by the FDA, and it is also due to their interesting biological properties that include, among others antibacterial, antifungal and antioxidant properties [133, 161].

Several investigations have focused on incorporating natural compounds such as antioxidants, antimicrobials, colorants, flavors, fortified nutrients, and spices in active packaging. One of the main objectives of this type of biologically active material is to allow controlled release on food surfaces. This method can serve as a better alternative to direct application of active compounds on food surfaces and slow penetration into the internal part of the food. [162]. Table 8.4 presents some studies on active films and coatings for food preservation.

Active packaging developed with different biopolymers such as chitosan [97, 136, 170], gelatin [134, 171], whey protein [165, 172], thermoplastic starch [167, 173], among others [168, 174–176], have been used as vehicles for active compounds, essential oils [172, 174, 177, 178] and vegetable and fruit extracts [97, 165, 179] obtained mainly from agro-industrial waste, mainly [180].

Table 8.4 Active films and coatings used in food preservation

Natural additive	Preservation method	Polymeric matrix	Property	Food application	Reference
Satureja khuzestanica essential oils	Nanoencapsulation	Chitosan	Antimicrobial and antioxidant activity	Lamb meat	[163]
Ginger essential oil	Nanoemulsion	Sodium caseinate	Antimicrobial and antioxidant activity	Chicken breast fillets	[164]
Rosemary and sage extract	Trapping – films	Whey protein concentrate	Antimicrobial activity	Soft cheese	[165]
Laurus nobilis and Rosmarinus officinalis essential oils	Encapsulation	Zein	Antimicrobial activity	Cheese slice	[166]
Ho wood and Cinnamon essential oils	Nanoencapsulation	Thermoplastic starch	Antifungal activity	Strawberry	[167]
Shallot onion wastes	Trapping – films	Sodium alginate and carboxymethyl cellulose	Anti-browning	Fresh-cut apple and potato	[168]
Akebia trifoliata (Thunb.) Koidz. peel extracts and montmorillonite	Trapping – films	Chitosan	Delaying crack and mature	A. trifoliata fruits	[169]

These active packaging and coatings have been used to preserve foods, mainly to increase the shelf life of the products and maintain their physicochemical and sensory characteristics mainly. Bahmid et al. [181] developed an antimicrobial cellulose acetate film containing finely ground mustard seeds (500 mg) was used to preserve low-fat ground beef, showing an increase in shelf life of 3.7 days compared to the sample without the film. Other studies developed in dairy products such as cheese, demonstrate the activity of gelatin-based film enriched with 20 μg ethanolic extract of *Lepidium sativum* seeds /mL reduced the syneresis by 40% and stabilized the color, peroxidation and bacteria growth as compared to the unwrapped sample after 6 days of storage [182]. Likewise, different active packaging for fruits and vegetables have been developed using natural additives such as extracts of *Artemisia scoparia* to maintain the quality parameters (microbial count, acidity loss, soluble solid content loss, weight loss and quality decay) of strawberries and loquats [183]. This is how the development of active packaging using various natural additives such as plant extracts and essential oils, mainly, are viewed as a potentially viable alternative for food preservation.

8.6 Conclusions

Different techniques focused on obtaining and preserving natural food additives (NFAs) have been used mainly in the food and pharmaceutical industries. Among the main techniques, the encapsulation is a widely used in the food industry to conserve NFAs, micronutrients, and bioactive compounds. This technique can be used to protect the degradation of these compounds in food processing, provide enhanced stability, prevent their interaction with other food components, mask undesirable sensory properties, and control release rate. To achieve efficient micro/nanoencapsulation, many factors must be considered: core material, carrier/matrix, and encapsulation technique. The selection of these factors directly affects the physicochemical properties of micro or nano-capsules, such as particle size, shape, cost, rate of release, and delivery of encapsulated compounds. Due to the growing importance of encapsulation processes in the food industry, new advances and innovations in this field have been developed in recent years, such as the combination of two encapsulation techniques or two or more carrier materials, co-encapsulation of active functional ingredients, and using of food by-products as a “green” wall material.

Another of the most used techniques and with a growing trend for the conservation of NFAs is by trapping. Different materials, preparation methods and structures in one, two, and three dimensions have allowed development systems to protect the bioactive compounds in nanostructures, hydrogels, and films through the physical interactions between the additive and the entrapment system. Future research studies could be addressed to improve the trapping efficiency and the thermal and chemical stability of bioactive molecules, as well as to apply these systems in food products. In addition, it is necessary to evaluate the functionality of bioactive molecules after the entrapment process.

Other aspects to consider in the use of these techniques are the low cost, the ease of design, the high performance, and the simplicity of the processes. In that case, adsorption processes make them an attractive tool for preserving, stabilizing, and removing/recovering a variety of NFAs. Adsorption shows several advantages over other preservation and separations techniques of bioactive compounds derived from selectivity, environmental impact, and toxicological effects. The large surface area materials such as clays, zeolites, and resins have been widely studied to preserve, recover, and purify NFAs. These materials have been shown an excellent performance in preserving NFAs properties along the time. Here, characteristics such as pH, temperature, light conditions, and many other variables in the adsorption of NFAs are essential, and they could drastically affect the process. The adsorption behaviors of NFAs components in the complex mixtures enable the possibility of obtaining concentrates enriched in defined NFAs, suitable for many applications in the food industry. Studies on the characterization of the detailed interactions between adsorbate and adsorbent at real simulated conditions, evaluating the influence of mentioned above variables, and on the regeneration and recycling of spent adsorbents are needed for design. Combining adsorption stages with other conventional and/or emerging technologies (encapsulation and trapping) provides opportunities to develop optimal, flexible, effective, and environmentally friendly processes for obtaining products with defined applications in the food industry.

Obtaining and preserving NFAs through encapsulation, trapping and absorption techniques has allowed their incorporation for the development of biodegradable packaging, films and coatings used in the food industry. The main NFAs used in this type of material are plant extracts, essential oils, which have demonstrated their effectiveness in food preservation, mainly due to their antimicrobial, antioxidant and anti-browning characteristics or due to the properties they exhibit, thus becoming a viable alternative for the total or partial substitution of polymers and synthetic preservatives that are in direct contact with food and that have sometimes presented adverse reactions in consumers

Conflicts of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Rangan C, Barceloux DG. Food additives and sensitivities. Dis Mon. 2009;55(5):292–311. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0011502909000157.
2. FDA. Essential oils, oleoresins (solvent-free), and natural extractives (including distillates). Substances generally recognized as safe. 2020. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=182.20.

3. McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K, et al. Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. Lancet. 2007;370(9598):1560–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673607613063.
4. Kus E, Eroglu HE. Genotoxic and cytotoxic effects of sunset yellow and brilliant blue, colorant food additives, on human blood lymphocytes. Pak J Pharm Sci. 2015;28(1):227–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25553699.
5. Panzella L, Moccia F, Toscanesi M, Trifuoggi M, Giovando S, Napolitano A. Correction: exhausted woods from tannin extraction as an unexplored waste biomass: evaluation of the antioxidant and pollutant adsorption properties and activating effects of hydrolytic treatments. Antioxidants 2019, 8, 84. Antioxidants. 2019;8(6):157. Available from: https://www.mdpi.com/2076-3921/8/6/157.
6. Hellwig V, Gasser J. Polyphenols from waste streams of food industry: valorisation of blanch water from marzipan production. Phytochem Rev. 2020;19(6):1539–46. Available from: http://link.springer.com/10.1007/s11101-020-09663-y.
7. Huang W, Feng Z, Aila R, Hou Y, Carne A, Bekhit AE-DA. Effect of pulsed electric fields (PEF) on physico-chemical properties, β-carotene and antioxidant activity of air-dried apricots. Food Chem. 2019;291:253–62. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814619306703.
8. Lee NY, Kim Y, Kim YS, Shin J-H, Rubin LP, Kim Y. β-Carotene exerts anti-colon cancer effects by regulating M2 macrophages and activated fibroblasts. J Nutr Biochem. 2020;82:108402. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0955286320304344.
9. Ribeiro D, Sousa A, Nicola P, Ferreira de Oliveira JMP, Rufino AT, Silva M, et al. β-Carotene and its physiological metabolites: effects on oxidative status regulation and genotoxicity in in vitro models. Food Chem Toxicol. 2020;141:111392. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0278691520302805.
10. Koop BL, Nascimento da Silva M, Diniz da Silva F, Thayres dos Santos Lima K, Santos Soares L, José de Andrade C, et al. Flavonoids, anthocyanins, betalains, curcumin, and carotenoids: sources, classification and enhanced stabilization by encapsulation and adsorption. Food Res Int. 2022;153:110929. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0963996921008292.
11. Zambrano-Zaragoza ML, Quintanar-Guerrero D, González-Reza RM. Nanocontainers in food preservation: techniques and uses. In: Smart nanocontainers. Elsevier; 2020. p. 137–55. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128167700000095.
12. Dong Y, Zhao M, Sun-Waterhouse D, Zhuang M, Chen H, Feng M, et al. Absorption and desorption behaviour of the flavonoids from Glycyrrhiza glabra L. leaf on macroporous adsorption resins. Food Chem. 2015;168:538–45. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814614011571.
13. Wang Z, Peng S, Peng M, She Z, Yang Q, Huang T. Adsorption and desorption characteristics of polyphenols from Eucommia ulmoides Oliv. leaves with macroporous resin and its inhibitory effect on α-amylase and α-glucosidase. Ann Transl Med. 2020;8(16):1004. Available from: http://atm.amegroups.com/article/view/50006/html.
14. Kammerer J, Carle R, Kammerer DR. Adsorption and ion exchange: basic principles and their application in food processing. J Agric Food Chem. 2011;59(1):22–42. Available from: https://pubs.acs.org/doi/10.1021/jf1032203.
15. Imam HT, Marr PC, Marr AC. Enzyme entrapment, biocatalyst immobilization without covalent attachment. Green Chem. 2021;23(14):4980–5005. Available from: http://xlink.rsc.org/?DOI=D1GC01852C.
16. Chopde S, Datir R, Deshmukh G, Dhotre A, Patil M. Nanoparticle formation by nano-spray drying & its application in nanoencapsulation of food bioactive ingredients. J Agric Food Res. 2020;2:100085. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2666154320300661.

17. Losini AE, Grillet AC, Bellotto M, Woloszyn M, Dotelli G. Natural additives and biopolymers for raw earth construction stabilization – a review. Constr Build Mater. 2021;304(July):124507. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0950061821022637.
18. Seibert JB, Bautista-Silva JP, Amparo TR, Petit A, Pervier P, dos Santos Almeida JC, et al. Development of propolis nanoemulsion with antioxidant and antimicrobial activity for use as a potential natural preservative. Food Chem. 2019;287(October 2018):61–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814619304017.
19. Caleja C, Barros L, Antonio AL, Carocho M, Oliveira MBPP, Ferreira ICFR. Fortification of yogurts with different antioxidant preservatives: a comparative study between natural and synthetic additives. Food Chem. 2016;210:262–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814616306422.
20. Sharma H, Mendiratta SK, Agrawal RK, Gurunathan K, Kumar S, Singh TP. Use of various essential oils as bio preservatives and their effect on the quality of vacuum packaged fresh chicken sausages under frozen conditions. LWT – Food Sci Technol. 2017;81:118–27. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0023643817302013.
21. Sezgin AC, Ayyıldız S. Food additives: colorants. Science within food: up-to-date advances on research and educational ideas. Formatex Research Center; 2017. p. 87–94. Available from: https://www.researchgate.net/publication/322404775_Food_Additives_Colorants.
22. Leroux J, Langendorff V, Schick G, Vaishnav V, Mazoyer J. Emulsion stabilizing properties of pectin. Food Hydrocoll. 2003;17(4):455–62. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0268005X03000274.
23. Surh J, Decker E, Mcclements D. Influence of pH and pectin type on properties and stability of sodium-caseinate stabilized oil-in-water emulsions. Food Hydrocoll. 2006;20(5):607–18. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0268005X05001530.
24. Hu Q, Gerhard H, Upadhyaya I, Venkitanarayanan K, Luo Y. Antimicrobial eugenol nanoemulsion prepared by gum arabic and lecithin and evaluation of drying technologies. Int J Biol Macromol. 2016;87:130–40. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0141813016301829.
25. Escobar S, Illanes A, Wilson L, Bernal C, Mesa M. In situ immobilization of β-galactosidase from Bacillus circulans in silica by sol-gel process: application in prebiotic synthesis. Eng Life Sci. 2016;16(4):396–404. Available from: https://onlinelibrary.wiley.com/doi/10.1002/elsc.201500097.
26. Song YJ, Yu HH, Kim YJ, Lee N-K, Paik H-D. The use of papain for the removal of biofilms formed by pathogenic Staphylococcus aureus and Campylobacter jejuni. LWT. 2020;127(March):109383. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0023643820303728.
27. dos Anjos MM, da Silva AA, de Pascoli IC, Mikcha JMG, Machinski M, Peralta RM, et al. Antibacterial activity of papain and bromelain on Alicyclobacillus spp. Int J Food Microbiol. 2016;216:121–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168160515301409.
28. Sudeep K, Upadhyaya J, Joshi DR, Lekhak B, Kumar Chaudhary D, Raj Pant B, et al. Production, characterization, and industrial application of pectinase enzyme isolated from fungal strains. Fermentation. 2020;6(2):59. Available from: https://www.mdpi.com/2311-5637/6/2/59.
29. Frankel EN. In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. Trends Food Sci Technol. 1993;4(7):220–5. Available from: https://linkinghub.elsevier.com/retrieve/pii/0924224493901554.
30. Alzate-Arbeláez AF, Dorta E, López-Alarcón C, Cortés FB, Rojano BA. Immobilization of Andean berry (Vaccinium meridionale) polyphenols on nanocellulose isolated from banana residues: a natural food additive with antioxidant properties. Food Chem. 2019;294:503–17. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814619308799.
31. Lau HH, Murney R, Yakovlev NL, Novoselova MV, Lim SH, Roy N, et al. Protein-tannic acid multilayer films: a multifunctional material for microencapsulation of food-derived bioac-

tives. J Colloid Interface Sci. 2017;505:332–40. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021979717306574.
32. Alexandre EMC, Lourenço RV, Bittante AMQB, Moraes ICF, Sobral PJ d A. Gelatin-based films reinforced with montmorillonite and activated with nanoemulsion of ginger essential oil for food packaging applications. Food Packag Shelf Life. 2016;10:87–96. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2214289416301491.
33. Bierhalz ACK, da Silva MA, Kieckbusch TG. Fundamentals of two-dimensional films and membranes. In: Biopolymer membranes and films. Elsevier; 2020. p. 35–66. Available from: https://linkinghub.elsevier.com/retrieve/pii/B978012818134800002X.
34. Zhao H, Yang Z, Guo L. Nacre-inspired composites with different macroscopic dimensions: strategies for improved mechanical performance and applications. NPG Asia Mater. 2018;10(4):1–22. Available from: http://www.nature.com/articles/s41427-018-0009-6.
35. Ganiari S, Choulitoudi E, Oreopoulou V. Edible and active films and coatings as carriers of natural antioxidants for lipid food. Trends Food Sci Technol. 2017;68:70–82. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0924224417302042.
36. Yuan E, Nie S, Liu L, Ren J. Study on the interaction of Hericium erinaceus mycelium polysaccharides and its degradation products with food additive silica nanoparticles. Food Chem X. 2021;12:100172. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2590157521000602.
37. Moraczewski K, Pawłowska A, Stepczyńska M, Malinowski R, Kaczor D, Budner B, et al. Plant extracts as natural additives for environmentally friendly polylactide films. Food Packag Shelf Life. 2020;26(October):100593. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2214289420306116.
38. Anuar H, Nur Fatin Izzati AB, Sharifah Nurul Inani SM, Siti Nur E'zzati MA, Siti Munirah Salimah AB, Ali FB, et al. Impregnation of cinnamon essential oil into plasticised polylactic acid biocomposite film for active food packaging. J Packag Technol Res. 2017;1(3):149–56. Available from: https://doi.org/10.1007/s41783-017-0022-1.
39. Hambardzumyan A, Foulon L, Bercu NB, Pernes M, Maigret JE, Molinari M, et al. Organosolv lignin as natural grafting additive to improve the water resistance of films using cellulose nanocrystals. Chem Eng J. 2015;264:780–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1385894714016143.
40. Dudu OE, Ma Y, Olurin TO, Oyedeji AB, Oyeyinka SA, Ogungbemi JW. Changes in structural and functional characteristics of cassava flour by additive complexations stimulated by hydrothermal conditions. Food Biosci. 2021;43(June):101289. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2212429221004144.
41. Cerruti P, Malinconico M, Rychly J, Matisova-Rychla L, Carfagna C. Effect of natural antioxidants on the stability of polypropylene films. Polym Degrad Stab. 2009;94(11):2095–100. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0141391009002687.
42. Tkaczewska J. Peptides and protein hydrolysates as food preservatives and bioactive components of edible films and coatings – a review. Trends Food Sci Technol. 2020;106:298–311. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0924224420306452.
43. Leandro GC, Capello C, Koop BL, Garcez J, Monteiro AR, Valencia A. Adsorption-desorption of anthocyanins from jambolan (Syzygium cumini) fruit in Laponite® platelets: Kinetic models, physicochemical characterization, and functional properties of biohybrids. Food Res Int. 2021;140(November 2020):109903.
44. Alexandra Y, Gaviria R, Stefanía N, Palencia N, Capello C. Nanostructured pH-indicator films based on cassava starch, Laponite, and Jambolan (Syzygium cumini) fruit manufactured by thermo-compression. Starch. 2021;2000208(73):1–11.
45. Ishwarya SP, Sandhya R, Nisha P. Advances and prospects in the food applications of pectin hydrogels. Crit Rev Food Sci Nutr. 2021;62(16):1–25. Available from: https://www.tandfonline.com/doi/full/10.1080/10408398.2021.1875394.
46. Varghese SA, Rangappa SM, Siengchin S, Parameswaranpillai J. Natural polymers and the hydrogels prepared from them. In: Hydrogels based on natural polymers.

Elsevier; 2020. p. 17–47. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128164211000021.
47. Gulrez SKH, Al-Assaf S, Phillips GO. Hydrogels: methods of preparation, characterisation and applications. In: Progress in molecular and environmental bioengineering-from analysis and modeling to technology applications. InTechOpen; 2011. p. 117–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20223627.
48. Du M, Lu W, Zhang Y, Mata A, Fang Y. Natural polymer-sourced interpenetrating network hydrogels: fabrication, properties, mechanism and food applications. Trends Food Sci Technol. 2021;116(May 2020):342–56. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0924224421004763.
49. Afzal S, Maswal M, Dar AA. Rheological behavior of pH responsive composite hydrogels of chitosan and alginate: characterization and its use in encapsulation of citral. Colloids Surf B Biointerfaces. 2018;169:99–106. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0927776518302789.
50. Huang J, Wang Q, Chu L, Xia Q. Liposome-chitosan hydrogel bead delivery system for the encapsulation of linseed oil and quercetin: preparation and in vitro characterization studies. LWT. 2020;117(July 2019):108615. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0023643819309570.
51. Bui HL, Huang C-J. Tough polyelectrolyte hydrogels with antimicrobial property via incorporation of natural multivalent phytic acid. Polymers (Basel). 2019;11(10):1721. Available from: https://www.mdpi.com/2073-4360/11/10/1721.
52. Akbari-Alavijeh S, Shaddel R, Jafari SM. Encapsulation of food bioactives and nutraceuticals by various chitosan-based nanocarriers. Food Hydrocoll. 2020;105(February):105774. Available from: https://doi.org/10.1016/j.foodhyd.2020.105774.
53. Ghorbanpour M, Bhargava P, Varma A, Choudhary DK. Biogenic nano-particles and their use in agro-ecosystems. In: Ghorbanpour M, Bhargava P, Varma A, Choudhary DK, editors. Biogenic nano-particles and their use in agro-ecosystems. Singapore: Springer Singapore; 2020. p. 1–606. Available from: http://link.springer.com/10.1007/978-981-15-2985-6.
54. Chawla R, Sivakumar S, Kaur H. Antimicrobial edible films in food packaging: current scenario and recent nanotechnological advancements- a review. Carbohydr Polym Technol Appl. 2021;2(December 2020):100024.
55. Rafiee Z, Nejatian M, Daeihamed M, Jafari SM. Application of different nanocarriers for encapsulation of curcumin. Crit Rev Food Sci Nutr. 2019;59(21):3468–97. Available from: https://www.tandfonline.com/doi/full/10.1080/10408398.2018.1495174.
56. Delfanian M, Sahari MA. Improving functionality, bioavailability, nutraceutical and sensory attributes of fortified foods using phenolics-loaded nanocarriers as natural ingredients. Food Res Int. 2020;137(July):109555. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0963996920305809.
57. Zhang C, Zhang X, Zhao G. Ferritin Nanocage: a versatile Nanocarrier utilized in the field of food, nutrition, and medicine. Nano. 2020;10(9):1894.
58. Peng S, Li Z, Zou L, Liu W, Liu C, McClements DJ. Enhancement of curcumin bioavailability by encapsulation in Sophorolipid-coated nanoparticles: an in vitro and in vivo study. J Agric Food Chem. 2018;66(6):1488–97. Available from: https://pubs.acs.org/doi/10.1021/acs.jafc.7b05478.
59. Dalponte Dallabona I, de Lima GG, Cestaro BI, Tasso I d S, Paiva TS, Laureanti EJG, et al. Development of alginate beads with encapsulated jabuticaba peel and propolis extracts to achieve a new natural colorant antioxidant additive. Int J Biol Macromol. 2020;163:1421–32. Available from: https://doi.org/10.1016/j.ijbiomac.2020.07.256.
60. Esmaili M, Ghaffari SM, Moosavi-Movahedi Z, Atri MS, Sharifizadeh A, Farhadi M, et al. Beta casein-micelle as a nano vehicle for solubility enhancement of curcumin; food industry application. LWT – Food Sci Technol. 2011;44(10):2166–72.

61. Pateiro M, Gómez B, Munekata PES, Barba FJ, Putnik P, Kovačević DB, et al. Nanoencapsulation of promising bioactive compounds to improve their absorption, stability, functionality and the appearance of the final food products. Molecules. 2021;26(6):1547.
62. Gulin-Sarfraz T, Kalantzopoulos G, Kvalvåg Pettersen M, Wold Åsli A, Tho I, Axelsson L, et al. Inorganic Nanocarriers for encapsulation of natural antimicrobial compounds for potential food packaging application: a comparative study. Nano. 2021;11(2):379. Available from: https://www.mdpi.com/2079-4991/11/2/379.
63. Timilsena YP, Wang B, Adhikari R, Adhikari B. Advances in microencapsulation of polyunsaturated fatty acids (PUFAs)-rich plant oils using complex coacervation: a review. Food Hydrocoll. 2017;69:369–81. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0268005X16306804.
64. Ghosh S, Sarkar T, Das A, Chakraborty R. Natural colorants from plant pigments and their encapsulation: an emerging window for the food industry. LWT. 2022;153:112527. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0023643821016807.
65. Comunian TA, Silva MP, Souza CJF. The use of food by-products as a novel for functional foods: their use as ingredients and for the encapsulation process. Trends Food Sci Technol. 2021;108:269–80. Available from: https://www.sciencedirect.com/science/article/pii/S0924224421000030.
66. Jyothi NVN, Prasanna PM, Sakarkar SN, Prabha KS, Ramaiah PS, Srawan GY. Microencapsulation techniques, factors influencing encapsulation efficiency. J Microencapsul. 2010;27(3):187–97. Available from: http://www.tandfonline.com/doi/full/10.3109/02652040903131301.
67. Saifullah M, Shishir MRI, Ferdowsi R, Tanver Rahman MR, Van Vuong Q. Micro and nano encapsulation, retention and controlled release of flavor and aroma compounds: a critical review. Trends Food Sci Technol. 2019;86:230–51. Available from: https://www.sciencedirect.com/science/article/pii/S0924224417307677.
68. Anandharamakrishnan C. Techniques for nanoencapsulation of food ingredients. In: Springerbriefs in food, health, and nutrition, vol. 8. New York: Springer; 2014. Available from: http://link.springer.com/10.1007/978-1-4614-9387-7.
69. Boostani S, Jafari SM. A comprehensive review on the controlled release of encapsulated food ingredients; fundamental concepts to design and applications. Trends Food Sci Technol. 2021;109:303–21. Available from: https://www.sciencedirect.com/science/article/pii/S0924224421000418.
70. Chaudhari AK, Singh VK, Das S, Dubey NK. Nanoencapsulation of essential oils and their bioactive constituents: a novel strategy to control mycotoxin contamination in food system. Food Chem Toxicol. 2021;149:112019. Available from: https://www.sciencedirect.com/science/article/pii/S0278691521000533.
71. Ephrem E, Najjar A, Charcosset C, Greige-Gerges H. Encapsulation of natural active compounds, enzymes, and probiotics for fruit juice fortification, preservation, and processing: an overview. J Funct Foods. 2018;48:65–84. Available from: https://www.sciencedirect.com/science/article/pii/S1756464618303177.
72. Smaoui S, Ben Hlima H, Ben Braïek O, Ennouri K, Mellouli L, Mousavi KA. Recent advancements in encapsulation of bioactive compounds as a promising technique for meat preservation. Meat Sci. 2021;181:108585. Available from: https://www.sciencedirect.com/science/article/pii/S0309174021001613.
73. Misra S, Pandey P, Mishra HN. Novel approaches for co-encapsulation of probiotic bacteria with bioactive compounds, their health benefits and functional food product development: a review. Trends Food Sci Technol. 2021;109:340–51. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0924224421000406.
74. Garavand F, Rahaee S, Vahedikia N, Jafari SM. Different techniques for extraction and micro/nanoencapsulation of saffron bioactive ingredients. Trends Food Sci Technol. 2019;89:26–44. Available from: https://www.sciencedirect.com/science/article/pii/S0924224418308719.

75. Kouamé KJE-P, Bora AFM, Li X, Sun Y, Liu L. Novel trends and opportunities for microencapsulation of flaxseed oil in foods: a review. J Funct Foods. 2021;87:104812. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1756464621004618.
76. Castro-Rosas J, Ferreira-Grosso CR, Gómez-Aldapa CA, Rangel-Vargas E, Rodríguez-Marín ML, Guzmán-Ortiz FA, et al. Recent advances in microencapsulation of natural sources of antimicrobial compounds used in food – a review. Food Res Int. 2017;102:575–87. Available from: https://linkinghub.elsevier.com/retrieve/pii/S096399691730621X.
77. Bao C, Jiang P, Chai J, Jiang Y, Li D, Bao W, et al. The delivery of sensitive food bioactive ingredients: absorption mechanisms, influencing factors, encapsulation techniques and evaluation models. Food Res Int. 2019;120:130–40. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0963996919301115.
78. de Moura SCSR, Berling CL, Germer SPM, Alvim ID, Hubinger MD. Encapsulating anthocyanins from Hibiscus sabdariffa L. calyces by ionic gelation: pigment stability during storage of microparticles. Food Chem. 2018;241:317–27. Available from: https://www.sciencedirect.com/science/article/pii/S0308814617314310.
79. Arriola NDA, Chater PI, Wilcox M, Lucini L, Rocchetti G, Dalmina M, et al. Encapsulation of stevia rebaudiana Bertoni aqueous crude extracts by ionic gelation – effects of alginate blends and gelling solutions on the polyphenolic profile. Food Chem. 2019;275:123–34. Available from: https://www.sciencedirect.com/science/article/pii/S0308814618316571.
80. Ortiz-Romero N, Ochoa-Martínez LA, González-Herrera SM, Rutiaga-Quiñones OM, Gallegos-Infante JA. Avances en las investigaciones sobre la encapsulación mediante gelación iónica: una revisión sistemática. TecnoLógicas. 2021;24(52):e1962. Available from: https://revistas.itm.edu.co/index.php/tecnologicas/article/view/1962.
81. Rodrigues FJ, Cedran MF, Bicas JL, Sato HH. Encapsulated probiotic cells: relevant techniques, natural sources as encapsulating materials and food applications – a narrative review. Food Res Int. 2020;137:109682. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0963996920307079.
82. Mudalip SA, Khatiman MN, Hashim NA, Che Man R, Arshad ZIM. A short review on encapsulation of bioactive compounds using different drying techniques. Mater Today Proc. 2021;42:288–96. Available from: https://www.sciencedirect.com/science/article/pii/S2214785321006349.
83. Nobari Azar FA, Pezeshki A, Ghanbarzadeh B, Hamishehkar H, Mohammadi M. Nanostructured lipid carriers: promising delivery systems for encapsulation of food ingredients. J Agric Food Res. 2020;2:100084. Available from: https://linkinghub.elsevier.com/retrieve/pii/S266615432030065X.
84. Assadpour E, Rostamabadi H, Jafari SM. Introduction to characterization of nanoencapsulated food ingredients. In: Jafari SM, editor. Nanoencapsulation in the food industry. Academic Press; 2020. p. 1–50. Available from: https://www.sciencedirect.com/science/article/pii/B9780128156674000018.
85. Pisoschi AM, Pop A, Cimpeanu C, Turcuş V, Predoi G, Iordache F. Nanoencapsulation techniques for compounds and products with antioxidant and antimicrobial activity – a critical view. Eur J Med Chem. 2018;157(5):1326–45. Available from: https://www.sciencedirect.com/science/article/pii/S0223523418307487.
86. Rashidinejad A, Bahrami A, Rehman A, Rezaei A, Babazadeh A, Singh H, et al. Co-encapsulation of probiotics with prebiotics and their application in functional/synbiotic dairy products. Crit Rev Food Sci Nutr. 2020;30:1–25. Available from: https://www.tandfonline.com/doi/abs/10.1080/10408398.2020.1854169.
87. Vaziri AS, Alemzadeh I, Vossoughi M, Khorasani AC. Co-microencapsulation of Lactobacillus plantarum and DHA fatty acid in alginate-pectin-gelatin biocomposites. Carbohydr Polym. 2018;199:266–75. Available from: https://www.sciencedirect.com/science/article/pii/S0144861718307835.
88. Marcillo-Parra V, Tupuna-Yerovi DS, González Z, Ruales J. Encapsulation of bioactive compounds from fruit and vegetable by-products for food application – a review. Trends Food

Sci Technol. 2021;116:11–23. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0924224421004477.
89. Marson GV, Saturno RP, Comunian TA, Consoli L, Machado MT d C, Hubinger MD. Maillard conjugates from spent brewer's yeast by-product as an innovative encapsulating material. Food Res Int. 2020;136:109365. Available from: https://www.sciencedirect.com/science/article/pii/S0963996920303902.
90. Mohammadian M, Waly MI, Moghadam M, Emam-Djomeh Z, Salami M, Moosavi-Movahedi AA. Nanostructured food proteins as efficient systems for the encapsulation of bioactive compounds. Food Sci Hum Wellness. 2020;9(3):199–213. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2213453020301385.
91. Artioli Y. Adsorption. In: Encyclopedia of Ecology. Elsevier; 2008. p. 60–5. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780080454054002524.
92. Kecili R, Hussain CM. Mechanism of adsorption on nanomaterials. In: Nanomaterials in chromatography. Elsevier; 2018. p. 89–115. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128127926000042.
93. Pourhakkak P, Taghizadeh M, Taghizadeh A, Ghaedi M. Adsorbent. In: Mehrorang G, editor. Adsorption: fundamental processes and applications. Elsevier; 2021. p. 71–210. Available from: https://www.sciencedirect.com/science/article/pii/B9780128188057000096.
94. Tien C. Adsorbents. In: Introduction to adsorption. Elsevier; 2019. p. 7–21. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128164464000026.
95. Capello C, Leandro GC, Maduro Campos CE, Hotza D, Mattar Carciofi BA, Valencia GA. Adsorption and desorption of eggplant peel anthocyanins on a synthetic layered silicate. J Food Eng. 2019;262:162–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0260877419302596.
96. Braida I, Mattea M, Cardarelli D. Extraction–adsorption–desorption process under supercritical condition as a method to concentrate antioxidants from natural sources. J Supercrit Fluids. 2008;45(2):195–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S089684460700304X.
97. Carvalho VVL, Gonçalves JO, Silva A, Cadaval TR, Pinto LAA, Lopes TJ. Separation of anthocyanins extracted from red cabbage by adsorption onto chitosan films. Int J Biol Macromol. 2019;131:905–11. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0141813019305926.
98. Khalesi H, Lu W, Nishinari K, Fang Y. Fundamentals of composites containing fibrous materials and hydrogels: a review on design and development for food applications. Food Chem. 2021;364(January):130329. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814621013352.
99. Zhang G, Sun Y, Guo Y, Liu J, Wu L, Lin J. The application of pomelo peel as a carrier for adsorption of epigallocatechin-3-gallate. J Sci Food Agric. 2018;98(11):4135–41. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jsfa.8931.
100. Bone S, Alum A, Markovski J, Hristovski K, Bar-Zeev E, Kaufman Y, et al. Physisorption and chemisorption of T4 bacteriophages on amino functionalized silica particles. J Colloid Interface Sci. 2018;532:68–76. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021979718308713.
101. Aliakbarian B, Casazza AA, Perego P. Kinetic and isothermal modelling of the adsorption of compounds from olive mill wastewater onto activated carbon. Food Technol Biotechnol. 2015;53(2):207–14. Available from: http://www.ftb.com.hr/images/pdfarticles/2015/April-June/ftb-53-207.pdf.
102. Chiou CT. Fundamentals of the adsorption theory. In: Partition and adsorption of organic contaminants in environmental systems. Hoboken: John Wiley & Sons, Inc.; 2003. p. 39–52. Available from: https://onlinelibrary.wiley.com/doi/10.1002/0471264326.ch4.
103. Soto ML, Moure A, Domínguez H, Parajó JC. Recovery, concentration and purification of phenolic compounds by adsorption: a review. J Food Eng. 2011;105(1):1–27. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0260877411000641.

104. Swenson H, Stadie NP. Langmuir's theory of adsorption: a centennial review. Langmuir. 2019;35(16):5409–26. Available from: https://pubs.acs.org/doi/10.1021/acs.langmuir.9b00154.
105. Sharif Nasirian V, Shahidi S, Tahermansouri H, Chekin F. Application of graphene oxide in the adsorption and extraction of bioactive compounds from lemon peel. Food Sci Nutr. 2021;9(7):3852–62. Available from: https://onlinelibrary.wiley.com/doi/10.1002/fsn3.2363.
106. Gao C, Zhao S, Yagiz Y, Gu L. Static, kinetic, and isotherm adsorption performances of macroporous adsorbent resins for recovery and enrichment of bioactive Procyanidins from cranberry pomace. J Food Sci. 2018;83(5):1249–57. Available from: https://onlinelibrary.wiley.com/doi/10.1111/1750-3841.14142.
107. Ribeiro HL, Oliveira AV d, Brito ES d, Ribeiro PRV, Souza Filho M d s M, Azeredo HMC. Stabilizing effect of montmorillonite on acerola juice anthocyanins. Food Chem. 2018;245:966–73. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814617318964.
108. Kohno Y, Kinoshita R, Ikoma S, Yoda K, Shibata M, Matsushima R, et al. Stabilization of natural anthocyanin by intercalation into montmorillonite. Appl Clay Sci. 2009;42(3–4):519–23. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0169131708001348.
109. Negm NA, Abd El Wahed MG, Hassan ARA, Abou Kana MTH. Feasibility of metal adsorption using brown algae and fungi: effect of biosorbents structure on adsorption isotherm and kinetics. J Mol Liq. 2018;264:292–305. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0167732218309875.
110. Rajabi H, Jafari SM, Feizi J, Ghorbani M, Mohajeri SA. Surface-decorated graphene oxide sheets with nanoparticles of chitosan-Arabic gum for the separation of bioactive compounds: a case study for adsorption of crocin from saffron extract. Int J Biol Macromol. 2021;186(July):1–12. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0141813021014732.
111. García-Pérez P, Losada-Barreiro S, Gallego PP, Bravo-Díaz C. Adsorption of gallic acid, propyl gallate and polyphenols from Bryophyllum extracts on activated carbon. Sci Rep. 2019;9(1):14830. Available from: http://www.nature.com/articles/s41598-019-51322-6.
112. Vazquez G, Alonso R, Freire S, Gonzalez Alvarez J, Antorrena G. Uptake of phenol from aqueous solutions by adsorption in a Pinus pinaster bark packed bed. J Hazard Mater. 2006;133(1–3):61–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0304389405006448.
113. Santos RR, Vermeulen S, Haritova A, Fink-Gremmels J. Isotherm modeling of organic activated bentonite and humic acid polymer used as mycotoxin adsorbents. Food Addit Contam Part A. 2011;28(11):1578–89. Available from: http://www.tandfonline.com/doi/abs/10.1080/19440049.2011.595014.
114. Kurozawa LE, Park KJ, Hubinger MD. Effect of maltodextrin and gum arabic on water sorption and glass transition temperature of spray dried chicken meat hydrolysate protein. J Food Eng. 2009;91(2):287–96. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0260877408004470.
115. Abdulmumeen HA, Risikat AN, Sururah AR. Food: its preservatives, additives and applications. Int J Chem Biomed Sci. 2012;1:36–47. Available from: https://www.researchgate.net/publication/268509774_Food_Its_preservatives_additives_and_applications.
116. Bearth A, Cousin M-E, Siegrist M. The consumer's perception of artificial food additives: influences on acceptance, risk and benefit perceptions. Food Qual Prefer. 2014;38:14–23. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0950329314000974.
117. Hou Y, Xie Z, Cui H, Lu Y, Zheng T, Sang S, et al. Trapping of glyoxal by propyl, octyl and dodecyl gallates and their mono-glyoxal adducts. Food Chem. 2018;269(April):396–403. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814618311683.

118. Gürses A. Natural products in adsorption technology. In: Integrating green chemistry and sustainable engineering. Hoboken: John Wiley & Sons, Inc.; 2019. p. 397–441. Available from: https://onlinelibrary.wiley.com/doi/10.1002/9781119509868.ch13.
119. Chen Q, Zhang TT, Jiang JG. Absorption and desorption behaviour of the flavonoids from Rubus chingii hu fruit on macroporous adsorption resins. Chiang Mai J Sci. 2018;45(1):331–41. Available from: https://www.thaiscience.info/Journals/Article/CMJS/10989296.pdf.
120. Pourhakkak P, Taghizadeh M, Taghizadeh A, Ghaedi M. Chapter 2 – Adsorbent. In: Mehrorang G, editor. Adsorption: Fundamental Processes and Applications. Elsevier; 2021. p. 71–210.
121. Villa CC, Valencia GA, Córdoba AL, Ortega-Toro R, Ahmed S, Gutiérrez TJ. Zeolites for food applications: a review. Food Biosci. 2022;46:101577. Available from: https://www.sciencedirect.com/science/article/pii/S2212429222000359.
122. Kohno Y, Asai S, Shibata M, Fukuhara C, Maeda Y, Tomita Y, et al. Improved photostability of hydrophobic natural dye incorporated in organo-modified hydrotalcite. J Phys Chem Solids. 2014;75(8):945–50. Available from: https://linkinghub.elsevier.com/retrieve/pii/S002236971400095X.
123. Kohno Y, Senga M, Shibata M, Yoda K, Matsushima R, Tomita Y, et al. Stabilization of flavylium dye by incorporation into Fe-containing mesoporous silicate. Microporous Mesoporous Mater. 2011;141(1–3):77–80. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1387181110003914.
124. Kohno Y, Inagawa M, Ikoma S, Shibata M, Matsushima R, Fukuhara C, et al. Stabilization of a hydrophobic natural dye by intercalation into organo-montmorillonite. Appl Clay Sci. 2011;54(3–4):202–5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0169131711002985.
125. Lima LCB, Silva FC, Silva-Filho EC, Fonseca MG, Zhuang G, Jaber M. Saponite-anthocyanin derivatives: the role of organoclays in pigment photostability. Appl Clay Sci. 2020;191:105604. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0169131720301691.
126. Zhang H, Zhang F, Yuan R. Applications of natural polymer-based hydrogels in the food industry. In: Hydrogels based on natural polymers. Elsevier; 2020. p. 357–410. Available from: https://linkinghub.elsevier.com/retrieve/pii/B978012816421100015X.
127. Ribeiro VR, Fernandes I d AA, Mari IP, Stafussa AP, Rossetto R, Maciel GM, et al. Bringing together Saccharomyces cerevisiae and bioactive compounds from plants: a new function for a well-known biosorbent. J Funct Foods. 2019;60(June):103433. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1756464619303573.
128. Qin X-S, Luo Z-G, Peng X-C. Fabrication and characterization of quinoa protein nanoparticle-stabilized food-grade Pickering emulsions with ultrasound treatment: interfacial adsorption/arrangement properties. J Agric Food Chem. 2018;66(17):4449–57. Available from: https://pubs.acs.org/doi/10.1021/acs.jafc.8b00225.
129. Tan C, Feng B, Zhang X, Xia W, Xia S. Biopolymer-coated liposomes by electrostatic adsorption of chitosan (chitosomes) as novel delivery systems for carotenoids. Food Hydrocoll. 2016;52:774–84. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0268005X15300618.
130. Cenobio-Galindo A d J, Díaz-Monroy G, Medina-Pérez G, Franco-Fernández MJ, Ludeña-Urquizo FE, Vieyra-Alberto R, et al. Multiple emulsions with extracts of cactus pear added in a yogurt: antioxidant activity, in vitro simulated digestion and shelf life. Foods. 2019;8(10):429. Available from: https://www.mdpi.com/2304-8158/8/10/429.
131. Carocho M, Ferreira ICFR. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol. 2013;51:15–25. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0278691512006941.
132. Alirezalu K, Pateiro M, Yaghoubi M, Alirezalu A, Peighambardoust SH, Lorenzo JM. Phytochemical constituents, advanced extraction technologies and techno-functional properties of selected Mediterranean plants for use in meat products. A comprehensive

review. Trends Food Sci Technol. 2020;100:292–306. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0924224420304398.
133. Cheikh D, Majdoub H, Darder M. An overview of clay-polymer nanocomposites containing bioactive compounds for food packaging applications. Appl Clay Sci. 2022;216(November 2021):106335. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0169131721003598.
134. Reyes LM, Landgraf M, Sobral PJA. Gelatin-based films activated with red propolis ethanolic extract and essential oils. Food Packag Shelf Life. 2021;27:100607. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2214289420306256.
135. Bonilla J, Sobral PJA. Investigation of the physicochemical, antimicrobial and antioxidant properties of gelatin-chitosan edible film mixed with plant ethanolic extracts. Food Biosci. 2016;16:17–25. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2212429216300499.
136. Bonilla J, Poloni T, Lourenço RV, Sobral PJA. Antioxidant potential of eugenol and ginger essential oils with gelatin/chitosan films. Food Biosci. 2018;23:107–14. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2212429217305497.
137. Giaconia MA, Ramos S d P, Pereira CF, Lemes AC, De Rosso VV, Braga ARC. Overcoming restrictions of bioactive compounds biological effects in food using nanometer-sized structures. Food Hydrocoll. 2020;107:105939. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0268005X20302757.
138. Bertolino M, Belviso S, Dal Bello B, Ghirardello D, Giordano M, Rolle L, et al. Influence of the addition of different hazelnut skins on the physicochemical, antioxidant, polyphenol and sensory properties of yogurt. LWT – Food Sci Technol. 2015;63(2):1145–54. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0023643815002674.
139. Martins A, Barros L, Carvalho AM, Santos-Buelga C, Fernandes IP, Barreiro F, et al. Phenolic extracts of Rubus ulmifolius Schott flowers: characterization, microencapsulation and incorporation into yogurts as nutraceutical sources. Food Funct. 2014;5(6):1091–100. Available from: http://xlink.rsc.org/?DOI=C3FO60721F.
140. Caleja C, Barros L, Antonio AL, Ciric A, Soković M, Oliveira MBPP, et al. Foeniculum vulgare Mill. as natural conservation enhancer and health promoter by incorporation in cottage cheese. J Funct Foods. 2015;12:428–38. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1756464614003958.
141. Caleja C, Barros L, Antonio AL, Ciric A, Barreira JCM, Sokovic M, et al. Development of a functional dairy food: exploring bioactive and preservation effects of chamomile (Matricaria recutita L.). J Funct Foods. 2015;16:114–24. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1756464615002066.
142. Zhao X, Cheng M, Wang C, Jiang H, Zhang X. Effects of dairy bioactive peptides and lotus seeds/lily bulb powder on flavor and quality characteristics of goat milk yogurt. Food Biosci. 2021;47:101510. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2212429221006350.
143. Abd El-Fat F, Hassan Sal H, Mosbah E-S, Samir E-SH, Abdel-Hady H. Utilization of natural antimicrobial and antioxidant of Moringa oleifera leaves extract in manufacture of cream cheese. J Biol Sci. 2018;18(2):92–106. Available from: https://www.scialert.net/abstract/?doi=jbs.2018.92.106.
144. Gabbi DK, Bajwa U, Goraya RK. Physicochemical, melting and sensory properties of ice cream incorporating processed ginger (Z ingiber officinale). Int J Dairy Technol. 2018;71(1):190–7. Available from: https://onlinelibrary.wiley.com/doi/10.1111/1471-0307.12430.
145. Pateiro M, Munekata PES, Sant'Ana AS, Domínguez R, Rodríguez-Lázaro D, Lorenzo JM. Application of essential oils as antimicrobial agents against spoilage and pathogenic microorganisms in meat products. Int J Food Microbiol. 2021;337:108966. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168160520304608.

146. Jayasena DD, Jo C. Essential oils as potential antimicrobial agents in meat and meat products: a review. Trends Food Sci Technol. 2013;34(2):96–108. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0924224413002033.
147. Šojić B, Pavlić B, Ikonić P, Tomović V, Ikonić B, Zeković Z, et al. Coriander essential oil as natural food additive improves quality and safety of cooked pork sausages with different nitrite levels. Meat Sci. 2019;157:107879. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0309174018312129.
148. Ghasemi G, Alirezalu A, Ghosta Y, Jarrahi A, Safavi SA, Abbas-Mohammadi M, et al. Composition, antifungal, phytotoxic, and insecticidal activities of Thymus kotschyanus essential oil. Molecules. 2020;25(5):1152. Available from: https://www.mdpi.com/1420-3049/25/5/1152.
149. Chivandi E, Dangarembizi R, Nyakudya TT, Erlwanger KH. Use of essential oils as a preservative of meat. In: Essential oils in food preservation, flavor and safety. Elsevier; 2016. p. 85–91. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780124166417000080.
150. Gómez B, Barba FJ, Domínguez R, Putnik P, Bursać Kovačević D, Pateiro M, et al. Microencapsulation of antioxidant compounds through innovative technologies and its specific application in meat processing. Trends Food Sci Technol. 2018;82:135–47. Available from: https://linkinghub.elsevier.com/retrieve/pii/S092422441830102X.
151. Carreón-Hidalgo JP, Franco-Vásquez DC, Gómez-Linton DR, Pérez-Flores LJ. Betalain plant sources, biosynthesis, extraction, stability enhancement methods, bioactivity, and applications. Food Res Int. 2022;151:110821. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0963996921007213.
152. Sugumar S, Ghosh V, Mukherjee A, Chandrasekaran N. Essential oil-based nanoemulsion formation by low- and high-energy methods and their application in food preservation against food spoilage microorganisms. In: Essential oils in food preservation, flavor and safety. Elsevier; 2016. p. 93–100. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780124166417000092.
153. Chuesiang P, Sanguandeekul R, Siripatrawan U. Phase inversion temperature-fabricated cinnamon oil nanoemulsion as a natural preservative for prolonging shelf-life of chilled Asian seabass (Lates calcarifer) fillets. LWT. 2020;125(September 2019):109122. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0023643820301109.
154. Aminzare M, Tajik H, Aliakbarlu J, Hashemi M, Raeisi M. Effect of cinnamon essential oil and grape seed extract as functional-natural additives in the production of cooked sausage-impact on microbiological, physicochemical, lipid oxidation and sensory aspects, and fate of inoculated Clostridium perfringens. J Food Saf. 2018;38(4):e12459. Available from: https://onlinelibrary.wiley.com/doi/10.1111/jfs.12459.
155. Tajik H, Farhangfar A, Moradi M, Razavi Rohani SM. Effectiveness of clove essential oil and grape seed extract combination on microbial and lipid oxidation characteristics of raw Buffalo Patty during storage at abuse refrigeration temperature. J Food Process Preserv. 2014;38(1):31–8. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1745-4549.2012.00736.x.
156. Khaleque MA, Keya CA, Hasan KN, Hoque MM, Inatsu Y, Bari ML. Use of cloves and cinnamon essential oil to inactivate Listeria monocytogenes in ground beef at freezing and refrigeration temperatures. LWT. 2016;74:219–23. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0023643816304455.
157. Ghaderi-Ghahfarokhi M, Barzegar M, Sahari MA, Azizi MH. Nanoencapsulation approach to improve antimicrobial and antioxidant activity of thyme essential oil in beef burgers during refrigerated storage. Food Bioprocess Technol. 2016;9(7):1187–201. Available from: http://link.springer.com/10.1007/s11947-016-1708-z.
158. Homayounpour P, Jalali H, Shariatifar N, Amanlou M, Khanjari A. Protective effect of Nanochitosan incorporated with free/nanoliposome cumin (Cuminum cyminum L.) aqueous extract on sardine fish. J Aquat Food Prod Technol. 2020;29(9):949–61. Available from: https://www.tandfonline.com/doi/full/10.1080/10498850.2020.1819497.

159. Shankar S, Karboune S, Salmieri S, Lacroix M. Development of antimicrobial formulation based on essential oils and gamma irradiation to increase the shelf life of boneless chicken thighs. Radiat Phys Chem. 2022;192:109893. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0969806X21005430.
160. Abdel-Wahab M, El-Sohaimy SA, Ibrahim HA, Abo El-Makarem HS. Evaluation the efficacy of clove, sage and kiwifruit peels extracts as natural preservatives for fish fingers. Ann Agric Sci. 2020;65(1):98–106. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0570178320300312.
161. Arroyo BJ, Santos AP, de Almeida de Melo E, Campos A, Lins L, Boyano-Orozco LC. Bioactive compounds and their potential use as ingredients for food and its application in food packaging. In: Bioactive compounds. Elsevier; 2019. p. 143–56. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128147740000086.
162. El-Saber Batiha G, Hussein DE, Algammal AM, George TT, Jeandet P, Al-Snafi AE, et al. Application of natural antimicrobials in food preservation: recent views. Food Control. 2021;126:108066. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0956713521002048.
163. Pabast M, Shariatifar N, Beikzadeh S, Jahed G. Effects of chitosan coatings incorporating with free or nano-encapsulated Satureja plant essential oil on quality characteristics of lamb meat. Food Control. 2018;91:185–92. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0956713518301543.
164. Noori S, Zeynali F, Almasi H. Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (Zingiber officinale) essential oil and its effect on safety and quality attributes of chicken breast fillets. Food Control. 2018;84:312–20. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0956713517304103.
165. Kontogianni VG, Kasapidou E, Mitlianga P, Mataragas M, Pappa E, Kondyli E, et al. Production, characteristics and application of whey protein films activated with rosemary and sage extract in preserving soft cheese. LWT. 2022;155:112996. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0023643821021496.
166. Göksen G, Fabra MJ, Ekiz HI, López-Rubio A. Phytochemical-loaded electrospun nanofibers as novel active edible films: characterization and antibacterial efficiency in cheese slices. Food Control. 2020;112:107133. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0956713520300499.
167. Ferreira RR, Souza AG, Quispe YM, Rosa DS. Essential oils loaded-chitosan nanocapsules incorporation in biodegradable starch films: a strategy to improve fruits shelf life. Int J Biol Macromol. 2021;188:628–38. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0141813021017050.
168. Thivya P, Bhosale YK, Anandakumar S, Hema V, Sinija VR. Development of active packaging film from sodium alginate/carboxymethyl cellulose containing shallot waste extracts for anti-browning of fresh-cut produce. Int J Biol Macromol. 2021;188:790–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0141813021016986.
169. Jiang Y, Yin H, Zhou X, Wang D, Zhong Y, Xia Q, et al. Antimicrobial, antioxidant and physical properties of chitosan film containing Akebia trifoliata (Thunb.) Koidz. peel extract/montmorillonite and its application. Food Chem. 2021;361:130111. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814621011171.
170. Duan M, Yu S, Sun J, Jiang H, Zhao J, Tong C, et al. Development and characterization of electrospun nanofibers based on pullulan/chitin nanofibers containing curcumin and anthocyanins for active-intelligent food packaging. Int J Biol Macromol. 2021;187:332–40. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0141813021015932.
171. da Nóbrega SE, Cesar de Albuquerque Sousa T, Cassiano de Santana Neto D, Brandão Grisi CV, Cardoso da Silva Ferreira V, Pereira da Silva FA. Edible active film based on gelatin and Malpighia emarginata waste extract to inhibit lipid and protein oxidation in beef patties. LWT. 2022;154:112837. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0023643821019903.

172. Esmaeili H, Cheraghi N, Khanjari A, Rezaeigolestani M, Basti AA, Kamkar A, et al. Incorporation of nanoencapsulated garlic essential oil into edible films: a novel approach for extending shelf life of vacuum-packed sausages. Meat Sci. 2020;166:108135. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0309174019309751.
173. Ortega-Toro R, Collazo-Bigliardi S, Roselló J, Santamarina P, Chiralt A. Antifungal starch-based edible films containing Aloe vera. Food Hydrocoll. 2017;72:1–10. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0268005X17305787.
174. Sarıcaoglu FT, Turhan S. Physicochemical, antioxidant and antimicrobial properties of mechanically deboned chicken meat protein films enriched with various essential oils. Food Packag Shelf Life. 2020;25:100527. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2214289420300909.
175. Saricaoglu FT, Turhan S. Performance of mechanically deboned chicken meat protein coatings containing thyme or clove essential oil for storage quality improvement of beef sucuks. Meat Sci. 2019;158:107912. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0309174019304115.
176. Bharti SK, Pathak V, Alam T, Arya A, Singh VK, Verma AK, et al. Materialization of novel composite bio-based active edible film functionalized with essential oils on antimicrobial and antioxidative aspect of chicken nuggets during extended storage. J Food Sci. 2020;85(9):2857–65. Available from: https://onlinelibrary.wiley.com/doi/10.1111/1750-3841.15365.
177. Zhang X, Wang H, Li X, Sun Y, Pan D, Wang Y, et al. Effect of cinnamon essential oil on the microbiological and physiochemical characters of fresh Italian style sausage during storage. Anim Sci J. 2019;90(3):435–44. Available from: https://onlinelibrary.wiley.com/doi/10.1111/asj.13171.
178. Vafania B, Fathi M, Soleimanian-Zad S. Nanoencapsulation of thyme essential oil in chitosan-gelatin nanofibers by nozzle-less electrospinning and their application to reduce nitrite in sausages. Food Bioprod Process. 2019;116:240–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0960308519302706.
179. Yang J, Hua S, Huang Z, Gu Z, Cheng L, Hong Y. Comparison of bioaccessibility of astaxanthin encapsulated in starch-based double emulsion with different structures. Carbohydr Polym. 2021;272:118475. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0144861721008626.
180. M. Rangaraj V, Rambabu K, Banat F, Mittal V. Natural antioxidants-based edible active food packaging: an overview of current advancements. Food Biosci. 2021;43:101251. Available from: https://linkinghub.elsevier.com/retrieve/pii/S221242922100376X.
181. Bahmid NA, Dekker M, Fogliano V, Heising J. Development of a moisture-activated antimicrobial film containing ground mustard seeds and its application on meat in active packaging system. Food Packag Shelf Life. 2021;30:100753.
182. Salem A, Jridi M, Abdelhedi O, Fakhfakh N, Nasri M, Debeaufort F, et al. Development and characterization of fish gelatin-based biodegradable film enriched with Lepidium sativum extract as active packaging for cheese preservation. Heliyon. 2021;7(10):e08099.
183. Hanif J, Khalid N, Khan RS, Bhatti MF, Hayat MQ, Ismail M, et al. Formulation of active packaging system using Artemisia scoparia for enhancing shelf life of fresh fruits. Mater Sci Eng C. 2019;100:82–93.

Chapter 9
Effect of Thermal Treatments on the Properties of Natural Food Additives

María Gabriela Goñi, María Celeste Pellegrini, and Alejandra Graciela Ponce

9.1 Introduction

Food processing often involves heat treatment, usually to increase sensory parameters such as flavor, texture, or color; and to improve safety and stability by reducing microbial population and inactivating endogenous enzymes [1]. In accordance with processing, food additives (FAs) play an important role in the food industry since their application can reduce processing costs, increase shelf life, and ensure safety [2].

Food processing in general, and thermal processing in particular, have increased the shelf life of several food products, most of the time with the aid of FAs [3]. Additives in food play important roles, like antioxidant, antibrowning, antimicrobial, color, and texture agents, among others [2]. There is extended evidence of the advantages of the use of FAs from the nutritional, functional, sensorial, economical point of view, and obviously from a safety point of view [2]. Some examples of the benefits of the use of FAs could be seen in bakery, meat processing, ready to eat food, and dairy industry [4–6].

Heat treatments used in the food industry, such as pasteurization, high temperature sterilization, drying, and evaporation, among others, ensure microbiological quality but favor the destruction of some food ingredients such as vitamins and agents with antioxidant activity such as heat-sensitive polyphenols [7]. Many FAs are especially heat sensitive and should be considered when a thermal process is

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included to obtain certain food products [8]. In the present chapter, this topic would be discussed, and current literature will be explored.

Nowadays, there is a growing interest in new natural food additives (NFAs) since consumers demand natural and environmentally friendly products [2]. As a consequence, several studies are focusing on finding potential ingredients in non-traditional sources such as residue from the citrus industry [9]. However, more research is needed in finding the effect of thermal treatment on these compounds when used as FAs in more complex foods.

NFAs often have a dual role in the food, including their technological aspect and nutritional or functional aspect, for example as polyphenolic compounds such as β-carotene which could act as colorant agent and antioxidant of lipids, as well as vitamins. Dietary fiber could be also used as a texturizing or gelling agent as well as a functional ingredient aimed to improve intestinal health. Intake of these types of bioactive compounds are often considered important to maintenance of health and for the prevention of non-transmissible diseases like cancer, cardiovascular illnesses, and several degenerative diseases [4, 8].

Generally, for a food to be safe and have a stable shelf life, the heat treatment that is applied must be prolonged, which promotes chemical and physical reactions that result in a food that is not healthy with detrimental effects on its quality. After heat treatment food may present strange odors or off-flavors associated with the loss of freshness and nutrients. This phenomenon is more common when solid or semi-solid foods are prepackaged in which the heat transfer when using conventional methods is slow and the temperature in the center of the product is difficult to rise [3]. Proper design of conventional or novel thermal processes requires a comprehensive understanding of the thermal properties of foods and quantitative changes of target microorganisms, enzymes, or quality attributes. It is desirable to select optimal process conditions to control microorganisms and enzymes while minimizing food quality degradations [3, 7, 10, 11].

Food matrices are complex systems, and they are greatly affected by thermal processing extensively reported and reviewed in the literature [12, 13]. FAs should also be considered, in order to assure their stability and therefore their functionality. Therefore, the aim of the present chapter was to comprehensively analyze and describe the effect of thermal processing on NFAs and to explore current alternatives and future needs on the subject.

9.2 Synthetic Versus Natural Food Additives: Pro and Cons

Codex Alimentarius defines a food additive as any substance that is not normally consumed by itself or as a common ingredient, regardless of its own nutritional value, but that is added into food with a technological, or organoleptic purpose. It could be included in the manufacturing, processing, packaging, storage, or any other step of the whole process. The FDA (Food and Drug Administration) in the United States has a similar definition as in most countries. Along with a definition,

most countries and organizations also have defined certain rules for their application and clear limits for their use in foods [2].

Nowadays, most consumers prefer foods added of NFAs, rather than synthetic food additives (SFAs) [9], which is seen by the food industry as an opportunity to find new and more efficient natural-based solutions, meanwhile fighting to reduce the overall use of SFAs, producing minimally processed goods [2, 7, 14]. The benefits of NFAs are endless, their synergy and effectiveness are a great leap over artificial additives that carry out, in most cases, only one effect on the food [2, 15].

Plant and fungi kingdoms are great sources of bioactive compounds that can be used to develop NFAs [2, 8, 15]. These natural compounds can be added as extracts, taking advantage of the synergistic effects between compounds, or as individual molecules, after purification, thus adding the most bioactive ones to the foodstuff. Although quite promising, NFAs still face some drawbacks and limitations. Therefore, an important research topic is the discovery of new alternative sources of NFAs fulfilling the different classes: preservatives (such as antimicrobials, antioxidants and antibrownings), nutritional additives, coloring agents, flavoring agents, texturizing agents, and miscellaneous agents. In accordance with consumer perception of NFAs, several new laws and requirements from private buyers are introducing changes in the allowed limits of several known additives, thus forcing the need for new natural alternatives [2].

9.3 Most Common Natural Food Additives

The antioxidant agents used as NFAs are substances that control the autoxidation of oils and fats by donating their hydrogen to free radicals originating in the initiation and propagation stages of autoxidation. Much research has been carried out using natural plant extracts in edible oils due to the tendency to minimize or avoid the use of SFAs. According to data in the literature, there are many natural antioxidants that can be extracted from inexpensive sources, such as most parts of the olive plant, green tea, sesame, medicinal plants, among others [16].

Natural plant extracts can assist in maintaining the appearance, taste, and quality of food without any negative impact on the color, odor, and taste profiles. A large variety of extracts have effective antioxidant and antimicrobial properties, constituting alternatives to conventional synthetic preservatives. Although most of the preservatives used in food are of synthetic origin, there are several products from naturally occurring plants that can be used as food preservatives. It is estimated that 1–10% of the 500,000 species of plants in the world, that is, approximately 500, have been used as food preservatives [17]. However, more research is needed in order to increase the number of food preservatives originated in plants, as extraction, purification, and stabilization of the extract are usually difficult. Moreover, its effectiveness is often limited to certain microbial populations and only acts as bacteriostatic or fungistatic agents while synthetic antimicrobials have larger targets.

9.4 Effect of Thermal Processing on Natural Food Additives

The stability of natural antioxidants to be used as NFAs is fundamental, mainly during thermal treatments. For example, it has been shown that most NFAs have higher antioxidant activity and thermal stability than SFAs in different edible oils [16].

Betalains are water-soluble nitrogenous pigments with coloring properties and antioxidant activities. These natural compounds have been incorporated into several foods. The stability of betalains is affected by different factors, such as temperature, pH, water activity, light, presence or absence of oxygen, and enzymatic action [18, 19]. Betacyanins in beet extracts have been noted as having pH stability in the range of 3–7 [20] and are readily susceptible to thermal degradation [21]. Temperatures above 50 °C are reported to produce color degradations and reduction in the antioxidant capacity. In the heat treatment, the betacyanins can be degraded by isomerization and/or decarboxylation [22]. A slight hypsochromic and hypochromic change can occur displacing the maximum absorption in the spectrum, therefore imparting an orange-red color [23]. Furthermore, betanin and isobetanin can be dehydrogenated and hydrolyzed causing the formation of neobetanin (4, 15-dehydrobetanin), which is bright yellow [19]. On the other hand, betaxanthins are also thermally sensitive and have lower stability than betacyanins [24].

Norbixin is a carotenoid with antioxidant properties, this compound is used as a natural colorant in various processed products; however, its chemical structure makes it susceptible to environmental factors such as light, oxygen, and temperature. A widely used technique to improve the stability and solubility of these compounds is microencapsulation. A study carried out by Tupuna et al. [25] showed that the efficiency of this technology depends on the encapsulation agents and the operating parameters. For this, a stability study was carried out using an aqueous model at temperatures of 60, 90, and 98 °C for 300 min. The thermal degradation kinetics of norbixinfollowed a first order kinetic reaction. The activation energy (E_a) required for the degradation of norbixin microcapsules (E_a = 15.08 kcal/mol) was twice that required for non-encapsulated norbixin (E_a = 7.61 kcal/mol). Microencapsulation by spray drying improved the thermal stability of norbixin. Therefore, the increases in the shelf life of this bioactive compound and norbixin microcapsules can be considered as an additive with high potential for use as a natural colorant in food and beverages [25].

Other technologies used to improve the stability of different NFAs, that is, the preservation of their functional properties, would be using encapsulation techniques, such as spray drying, spray cooling, coacervation, extrusion, fluidized bed coating and polymerization. Microencapsulation is described as a technique to trap small particles of solids or droplets of liquids or gases in a biopolymer to result in small spheres called microcapsules or microparticles with diameters ranging from 1 to 1000 μm. This technique could simplify the manufacture, handling and storage of food, reducing production costs. In addition, bioactive compounds microencapsulated are protected against environmental conditions, thus improving their stability [26]. On the other hand, nanoencapsulation process, for example, is an interesting

and promising method applied by the food industry to obtain special NFAs with antimicrobial and antioxidant properties and high thermal stability [27]. The encapsulation of bioactive substances protects their active molecules from thermal degradation processes and improves their physical and chemical stability and solubility in foods (e.g., solubilization of hydrophilic components and hydrophobic matrices and vice versa) [28].

Other bioactive agents with potential application to be used as NFAs are essential oils that have antimicrobial activity since they are generally non-toxic and have important properties for food preservation. In addition, some of them have the property of being antioxidants and, when they are protected by coatings such as nanocapsules, they can be used to produce high-performance antimicrobial or antioxidant active packaging. Understanding the content of bioactive compounds that are part of the food is crucial for the formulation of new dietary plans. This information is also important for consumers with progressive development of nutritional awareness [29].

9.4.1 Blanching

Blanching is a widely used process in food industries that process vegetables and some fruits. This treatment is part of a stage prior to other processes, whose main objective is to inactivate enzymes, increase the fixation of chlorophyll (especially important in green vegetables) and soften the product to favor its subsequent packaging. Blanching is prior to freezing, which seeks the destruction of enzymes that affect color, flavor, and vitamin content. In the blanching process foods are heated at temperatures between 70 and 100 °C for a time between 30 s and 3 min, then foods are cool down. Otherwise, this process contributes to the proliferation of thermophilic microorganisms, resistant to temperature [30].

There are two widely distributed enzymes in plants that are resistant to heat: peroxidase and catalase. Verifying the absence of their activity is a clear indicator of the effectiveness of blanching. Sicari et al. [31] conducted a study with the aim of investigating the phytochemical content and bioactivity of traditionally consumed wild plants (*Hypochaeris laevigata*, *Hypochaerisradicata*, *Hyoseris radiata* and *Hyoserislucida* subsp. *taurina*), both fresh as after blanching. The impact of processing on these food matrices was evaluated. Among the bioactive phytochemicals, Total Phenols Content (TPC), Total Flavonoids Content (TFC), lycopene, β-carotene and chlorophylls were quantified. The samples were studied for their antioxidant potential using different approaches and as inhibitors of enzymes related to obesity and hyperglycemia. Fresh and scalded samples, as well as residual blanching water, were studied. Blanching determined a reduction of up to 45% in the content of all classes of phytochemicals investigated. On the other hand, it was observed that the blanching water retained most of the bioactive compounds being correlated with a good antioxidant and inhibitory activity against enzymes linked to obesity and related diseases such as type 2 diabetes [31].

In another research, Otálora et al. [32] studied the thermal stability at 5, 25, and 45 °C of betalains present in by-products of blanching and cutting Beta vulgaris tissues. The blanching water degraded pigments independently of the temperature studied. Red beet powders underwent thermal degradation of pigments only at 45 °C. This can be attributed to its low water activity and the presence of lignin which can protect the pigments from thermal degradation, through its antioxidant activity, allowing these powders to be used as a food colorant up to 45 °C. Chromatographic studies showed that storage at 45 °C for 6 days affected the chemical stability of betalains. Degradation reactions may affect the use of these powders as natural pigments in heat-treated foods at temperatures above 45 °C after the inclusion of the pigment in the food formulation [26].

Blanching is a very important unit operation in fruits and vegetable processing. It not only affects the inactivation of polyphenol oxidase (PPO) and peroxidase (POD) but also affects other quality attributes of products such as microbiological quality, color, and texture. Blanching can inactivate enzymes present in products, enhancing dehydration rate, removing pesticide residue, and reducing microbial load. The indicators that are frequently used to assess include POD and PPO enzymes, ascorbic acid and nutrient contents, color, and texture. The conventional water and steam blanching methods are mature technologies that are being applied in many food processors [33].

9.4.2 Pasteurization and Sterilization

Pasteurization and sterilization are the most used techniques to inactivate enzymes and microorganisms in food products, increasing the food shelf life [34]. Thermal pasteurization is mainly used for the manufacture of microbiologically safe food products, by reducing or inactivating the microbial count [35], but due to the high temperature required, it can affect the organoleptic and nutritional properties in some foods compared to unconventional technologies [36]. Sterilization is considered one of the most effective techniques in food preservation since it can provide almost complete inactivation of microorganisms, including spores, which leads to products having a longer shelf life [37, 38], but this, like pasteurization, can in some cases affect the nutritional properties mainly of antioxidant compounds including vitamins and induce changes in color, flavor, and texture which affect the sensory quality of the food. Undesirable by-products can even be formed during processing, affecting the quality of the final product [37, 39].

The use of NFAs in pasteurized milk processing allows enhancing the functionality of this product. In this way, matoa (*Pometia enhancing*) and alginate have been used as ingredients that exerts antimicrobial properties against *Staphylococcus aureus* and *Escherichia coli*. Triana et al. [40] investigated the antimicrobial activity of matoa leaf extract and alginate. The authors concluded that concentrations of 0.20% and 0.2–0.3% in the use of matoa leaf extract and alginate respectively had the best inhibitory effects on the growth of *Escherichia coli* and *Staphylococcus*

aureus. Milk was added with matoa leaf extract and alginate at particular concentrations, then pasteurized using high-temperature, short-time (HTST) at 72 °C for 15 s. Finally, authors observed that supplementation of matoa leaf extract and alginate in pasteurized milk was able to exert inhibitory effects against *Sthaphylococcus aureus* and *Escherichia coli*.

On the other hand, phytosterols are plant sterols recommended as NFAs for hypercholesterolemia, and tocopherols are well-known antioxidants. However, temperature sensitivity, lipophilicity, and efficacy are formulation dependent. Poudel et al. [41] studied the development of liposomes containing brassicasterol, campesterol, and β-sitosterol obtained from canola oil deodorant distillate, along with alpha, gamma, and delta tocopherol. Three types of formulations were produced: thin-film hydration-homogenization, thin-film hydration-ultrasonication, and the Mozafari method which consists of one of the most recently introduced and one of the simplest techniques for the preparation of liposomes and nanoliposomes and allows manufacture of carrier systems in one-step, without the need for the prehydration of ingredient material, and without employing toxic solvents or detergents. The stability of the liposomal formulations before and after pasteurization using the HTST technique was investigated for 1 month. Liposomes with optimal particle size (less than 200 nm) and zeta potential (−9 to −14 mV) were incorporated into orange juice, showing adequate stability after pasteurization (72 °C for 15 s) for 1 month stored at 4 °C.

In another research, Noriega et al. [42] determined the nutritional value and safety of a puree using NFAs and modified some operational parameters in the conventionally used process. For the food formulation, the following ingredients were used: chicken breast, potatoes, carrots, leeks, tomatoes, olive oil, and mineral water. The NFAs selected for the preparation of the formulated product were orange juice due to its high content of vitamin C and, therefore, high antioxidant capacity, and leek due to its antifungal property, constituting a nutritious and healthy natural alternative to avoid SFAs such as sorbates and benzoates. In the methodology, two important sterilization factors were adjusted (temperature and time evacuation), taking into account the effect on the nutritional and organoleptic properties, as well as the microbiological safety of the product. Two sterilization methods were applied using the same temperature (121.1 °C) and different times (40 and 45 min), to propose the variation of the sterilization regimes and to use times shorter than those usually recommended. The obtained nutritional values reported as g per 100 g (7.8 of proteins, 2.6 of fat, 20.2 of carbohydrates, 6.2 of fiber, and energy) for the mashed vegetables and chicken preserved, heated for40 min of sterilization were superior to those obtained in foods heated for 45 min, indicating that the nutritional content was better at lower sterilization time. The microbiological analysis shows that the products obtained do not present microorganisms selected as safety indicators (total coliforms, *Escherichia coli* and molds and yeast). Finally, from the sensory evaluation it was established that the sample subjected to the sterilization at 121.1 ° C for 40 min had greater acceptance in the respondents for its organoleptic characteristics [42].

Both pasteurization and sterilization are based on time–temperature combination processes applied to food products to achieve intended target lethality [43].

9.4.3 Drying

Drying is a preservation technology that reduces the moisture content of foods, thus reducing the weight and volume and facilitating the transport and storage of the product. When fruits and vegetables are dried, the water activity decreases (<0.6), which inhibits the development of spoilage microorganisms and enzyme activity, as well as physical and chemical changes during storage [44].

Drying is usually defined as the thermal substance removal process of volatiles (moisture) until obtaining a dry product. It is a unit operation in which the simultaneous transport of heat and mass occurs: the transfer of energy (mainly as heat energy) from the surrounding medium to evaporate moisture from the surface and the transfer of internal moisture to the surface of the solid and its evaporation later. The removal of water in the form of vapor from the surface of the solid depends on external conditions of temperature, flow, and humidity of the air, the surface area of the exposed material and pressure while the movement of water through the solid depends on its physical composition, the temperature, and its percentage of humidity. Energy transfer can occur by convection, conduction, or radiation, or in some cases by a combination of them [45].

Corrêa-Filho et al. [46] investigated the microencapsulation of β-carotene in arabic gum using the spray drying method. The arabic gum concentration and drying inlet temperature influenced the drying yield, encapsulation efficiency, and load capacity responses. The antioxidant activity of β-carotene was reduced from 2.35 μmol Trolox/mg of β-carotene to 0.78 μmol Trolox/mg of β-carotene when microencapsulated at high temperature (200 °C) in relation to low temperature (110 °C).

Drying technology is an alternative to consuming fresh fruit and vegetables, which allows their use out of season. The use of dehydrated can be multiple: preparation of red food dyes to enhance the color of ice cream, tomato puree, desserts, jams and jellies, sauces, sweets, and cereals, as well as in dry forms such as chips, tea, bakery powder, food supplements, etc. Dehydration alters the properties of the food and therefore the color and reflectance. NFAs such as carotene or chlorophyll undergo changes caused by heat during drying, such as oxidation. In general, pigment degradation can be observed with temperature and time increasing during the drying process. Among the non-enzymatic browning reactions are Maillard and caramelization reactions. The Maillard reaction involves amino acids and reducing sugars that give rise to the formation of melanoidins, with the consequent loss of nutritional value. These reactions start at 70 °C and their rate depends on the type of sugars present in the food. Furthermore, foods with a water activity in the range of 0.5 and 0.8 are more susceptible to non-enzymatic browning. Caramelization reactions occur with sugars at temperatures above 120 °C, giving rise to dark products called caramels [47].

The development of powder blends using spray drying of avocado as a powdered drink is an attractive option that has generated products with high nutritional value and stability. Dantaset al [48] used an experimental design to evaluate the influence of drying conditions on avocado formulations. To execute this, work an industrial

spray drying unit with a capacity to evaporate 10 kg of water per hour was available for product production. The experimental points were selected so the outlet temperature would be at least 10 °C below the estimated glass transition temperature. Atomization gas flow rate (Fatom) varying between 2 to 4 kg/h was combined with drying gas inlet temperature (Tin) varying between 80 to 120 °C. A final center point of 3 kg/h for Fatom and 100 °C for Tin was used. Results revealed that inlet temperature in combination with smaller droplets the primary factors in process yield and setting powder properties such as moisture content and water activity. This combination suggests that higher evaporation rates are responsible for a smooth and optimized process. Besides inlet temperature and atomization flow rate, maltodextrin proved to be essential for the spray drying of avocado. The inclusion of maltodextrin preserved protein, ascorbic acid, and phenolic compounds during the drying process, possibly due to the stabilization of these compounds by hydrogen bonds.

9.4.4 Frying

Frying is a cooking technique used to prepare sensory-friendly foods that are primarily characterized by a crispy crust, moist center, and appealing flavor. In frying, food is immersed in hot oil (150–190 °C), causing mass exchange and heat transfer between the frying oil and the fried food. Under deep frying conditions, the oil which is in contact with oxygen and moisture from the food can be hydrolyzed, oxidized, or even degraded thermically [49]. There are numerous publications that indicate that frying causes the formation of undesirable and harmful compounds resulting in food of less quality [49]. Frying in olive oil can lead to the incorporation of bioactive compounds from the oil in the food, decreasing the food oxidation and positively affecting the nutritional properties of the fried food [50].

Recently, Carvalho et al. [50] carried out an investigation on the evolution of the profile of virgin olive oil (VOO) during consecutive frying cycles and evaluated the transfer of metabolites to French fries. In this study, the evolution of 56 compounds was monitored by two complementary methods using liquid chromatography, mass spectrometry, diode array and fluorescence detectors. Sterols and lignans were remarkably stable (greater than 70% retention in frying oil). Seven of the ten classes of compounds identified in the oil transferred to the fry. French fries in arbequina oil from Brazil incorporated the highest amounts of minor components of virgin olive oil among the analyzed samples, and sterols presented the highest transfer rate. French fries were enriched with bioactive compounds from olive oil during frying, especially in the first 2 days, improving their nutritional value.

Frying is a technology used in foods rich in starch. After ingestion, starch and fat in food are hydrolyzed by enzymes in the human digestive tract, thus providing an important source of energy (glucose and fatty acids) for the human body. On the other hand, excessive consumption of rich foods in fried starches can promote overweight, obesity, and other chronic diseases. In addition, frying can generate toxic

products that harm people's health [51]. Because of this, there is great interest in developing alternative frying technologies that reduce the levels of undesirable components in fried foods, such as vacuum, microwave, air, and radiation frying methods. Vacuum frying is one of the earliest and most mature alternative frying methods, still has some problems that are difficult to solve, such as long processing times, the production of "off" flavors, and high equipment costs. However, the utilization of a combination of microwave and ultrasonic waves can be used to overcome these problems to some extent. These results suggest that it is feasible to improve the efficacy of alternative frying methods by combining different technologies. As a result, it may be possible to improve the quality and nutritional value of fried starchy products, as well as develop new products. Nevertheless, a lot of research is still needed to clarify the physicochemical mechanisms involved in different innovative frying technologies and to create commercially viable processing operations. The cost of air-frying is relatively low, and it has already entered the kitchen of many ordinary families, but there are still some problems with the taste of the products produced using this method [51].

On the other hand, Sordini et al. [52] evaluated the effect of a phenolic extract from oil mill wastewater on the stabilization of refined olive oil and the quality of French fries during the frying process. Frozen pre-fried potatoes were fried at 180 °C for 8 min in refined olive oil enriched with different concentrations of a phenolic extract, while oil enriched with a common synthetic antioxidant (butylated hydroxytoluene) was used for comparison. The frying process was carried out for 6 h. The phenolic extracts were mixed into two samples of refined olive oil (1.6 kg each) to reach final phenolic compound concentrations of 400 and 600 mg/kg, expressed as the sum of tyrosol (p-HPEA), hydroxytyrosol (3,4-DHPEA), the dialdehydic form of decarboxymethylelenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) and verbascoside. The phenolic extract has been revealed as a very promising oil stabilizing agent during frying, playing an important role (dose-dependent) in the preservation of antioxidants both in the oil and in the food, in the reduction of the formation of unwanted compounds (acrolein and hexanal), and in contrasting the production of acrylamide. In this way, the phenolic extract can be used as a source of natural antioxidants to replace (or avoid) SFAs in oils.

In order to offer consumers quality products that meet their expectations and satisfaction and, in turn, improve their quality characteristics, a new paradigm is required. More research is needed to propose that fried foods need not be a health risk in a balanced diet when frying technology and oil quality are carefully maintained.

9.4.5 Microwave

Microwave technology is a form of electromagnetic radiation where wavelengths in the range of 300 and 300,000 MHz exerts their effect by inducing friction in the polar molecules of food, thus generating considerable amounts of heat, requiring for

this, shorter exposure times compared to conventional heating; in which heat transfer occurs through a temperature gradient. Microwave heating of foods is an attractive process due to the rapid temperature rise, controllable heat deposition, and easy cleaning opportunities. This method has been used to pasteurize and sterilize with advantages over conventional heating for the basic reason that the process is fast and requires the shortest time to reach the desired process temperature. The dielectric heating mechanism is different from traditional conduction heating, and itis used on polar molecules and charged ions that interact with the alternating electromagnetic fields, resulting in rapid and volumetric heating through their frictional losses. Such a heating pattern would cause a certain change in the microwave treatment, which is an indisputable reality [43].

Aiming to apply the microwave technology in foods, Shang et al. [53] investigated the effects of microwave-assisted extraction conditions on antioxidant capacity of sweet tea (*Lithocarpus polystachyus Rehd.*) and identified its antioxidants. In this study, the optimization of parameters for the extraction of antioxidants was: ethanol concentration of 58.43% (v/v), solvent to sample ratio of 35.39:1 mL/g, extraction time of 25–26 min, extraction temperature of 50 °C and microwave power of 600 W. In addition, the largest antioxidant components in the extract were detected by high performance liquid chromatography with diode array detection (HPLC-DAD), including phlorizin, phloretin, and trilobatin. Under this optimal condition, the ferric reducing antioxidant power value of the extract was 381.29 ± 4.42 μM Fe(II)/g dry weight, the Trolox equivalent antioxidant capacity value was 613.11 ± 9.32 μM Trolox/g dry weight and the total phenolic content value was 135.94 ± 0.52 mg gallic acid equivalent /g dry weight. The authors concluded that the obtained extract could be used as a food additive or become a functional food for the prevention and treatment of diseases related to oxidative stress.

Depending on the case, boiling is preferred over other heat treatments. As in the case of potato tubers, boiling (1 h) was chosen as the best treatment over microwave cooking (20 min at highest power level) and baking (204 °C for 1 h) methods, because boiled potato tubers retained more polyphenols (flavonols, lutein, andanthocyanins) [54]. Few investigations suggest that the use of high temperatures increases the diffusion of the solvent in the food matrix and results in a higher yield, the non-target material can also be extracted together with the required material. On the other hand, high temperatures and longer extraction time sometimes cause degradation of the target material [55].

Hayatet al [56]. concluded that the phenolic content of pomace extracts was higher at higher microwave powers, with yields of 1163–1317 μg/g dry weight and 664–854 μg/g dry weight applying powers of 125–500 W respectively. These results suggest that greater amounts of phenolic compounds can be achieved as microwave power was increased.

In vegetables, the application of microwave treatments produces a localized increase in temperature that results in tissue alteration, causing the displacement of phenolic compounds to the surrounding solvents [57].

While microwave ovens are commonly used to heat and reheat food, trends show that they are increasingly being used for cooking and defrosting as well. The

application of this technology in industries has increased in recent years and microwave processing units have been developed on an industrial scale for drying, precooking meat, pasteurizing prepared meals, and tempering meat and fish. Modern food consumers demand high-quality, minimally processed products, which has led to the development of new microwave processing technologies for microwave-assisted thawing, blanching, baking, pasteurization, and bioactive compound extraction [58].

9.4.6 Ohmic Heat (OH) Processing

Ohmic heating occurs when an electric current passes through the food, causing the temperature inside to rise as a result of the resistance it offers to the passage of the electric current. The advantages of this process derive from the fact that the heating takes place inside the food. In this way, and unlike what happens in conventional heating, there are no hot contact surfaces. Ohmic heating is fast and has greater penetration capacity than microwaves, which makes it especially useful in the case of particulate foods, sauces, fruit purees, liquid eggs, or meat products, among others. This type of treatment prevents overheating, which allows less deterioration in the constituents and less formation of deposits, the latter aspect of special relevance in foods rich in salts and proteins, such as milk [59].

There are a large number of applications for ohmic heating including blanching, pasteurization, sterilization, thawing, evaporation, dehydration, fermentation, and extraction, among others. A difference with respect to microwaves is the absence of equipment in the domestic sphere. They do exist at the scale of pilot plants and industry.

Unlike conventional technologies, the application of electric fields during ohmic heating induces a more rapid inactivation of lipoxygenase and polyphenol oxidase. Thus, ohmic heating was found to be more efficient for the required pectin esterase and microbial inactivation due to a shorter treatment time. Compared to conventional pasteurization, ohmic treatment, the flavor compounds are not degraded as quickly and better products with higher quality are produced compared to those produced by conventional food processing technologies [55]. On the other hand, this technology has several advantages compared to conventional heating, such as providing a higher yield, maintaining the higher nutritional value of food, faster and more uniform heating of food, it is also cleaner and more environmentally friendly [60].

Ohmic heating has shown higher extraction yields of different substances such as beet dye, sucrose, oil and other bioactive substances from rice bran and red polyphenols, grape pomace [61]. In raw artichokes, the total phenolic content expressed was 1639.45 ± 15.34 mg GAE/100 g of fresh food product. In the case of blanched food samples, this processing technology significantly affected the residual contents of total phenolic compounds. Particularly with respect to the raw samples, ohmic heat processing resulted in a 29% increase in the concentration of total polyphenols. In

contrast, in artichokes, immediately after conventional treatment, a 27% increase in the concentration of total phenolic compounds was observed [62].

The optimization of the different parameters to apply this technology to different food matrices requires further study. Therefore, it is necessary to understand, characterize and model this phenomenon in order to optimize and possibly exploit its effects.

9.5 Future Aspects

Currently, the most investigated techniques that do not require strong thermal treatments are: high hydrostatic pressure, pulsed electric fields, ionizing radiation, ultrasound, microwaves, cold plasma, among others. Which are capable of inactivating microorganisms at room temperature or at not very high temperatures, preventing defects caused by heat [36] in order to produce safer foods and minimize losses and waste caused by the food deterioration, especially fruit and vegetables which are highly perishable. New research in those areas of vacancy could be useful in the next few years, in order to answer the market demand for better alternatives.

Exploring new sources of NFAs, such as bioactive compounds obtained from plant processing, from fungi or algae, could also be relevant to a growing demand. It could lead to new antioxidant or antimicrobial compounds, as well as to improve sustainability by reducing pollution and adding value to existing by-products.

9.6 Conclusions

Traditional heat treatments are essential for the food industry and guarantee the required safety, prolonging the shelf life of food. However, these treatments cause loss of desired organoleptic properties and damage to temperature-labile nutrients and vitamins. Because of this, new thermal and non-thermal technologies have emerged to meet the required demands for the safety or shelf life of food products while minimizing the effects on the nutritional and quality attributes of a product. The validation of these processes in the industry has become a challenge, considering the complexities of food matrices and the variety of foods produced. These technological drivers for validation are shelf-life extension, nutritional and sensory aspects, new functional and organoleptic properties, consumer acceptability and environmental impact.

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References

1. Deb-Choudhury S, Cooney J, Brewster D, Clerens S, Knowles SO, Farouk MM, et al. The effects of blanching on composition and modification of proteins in navy beans (Phaseolus vulgaris). Food Chem. 2021;346:128950.
2. Carocho M, Morales P, Ferreira ICFR. Natural food additives: Quo vadis? Trends Food Sci Technol. 2015;45:284–95.
3. Ling B, Tang J, Kong F, Mitcham EJ, Wang S. Kinetics of food quality changes during thermal processing: a review. Food Bioprocess Technol. 2015;8(2):343–58.
4. Ajila CM, Leelavathi K, Prasada Rao UJS. Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. J Cereal Sci. 2008;48(2):319–26.
5. Vergara-Valencia N, Granados-Pérez E, Agama-Acevedo E, Tovar J, Ruales J, Bello-Pérez LA. Fibre concentrate from mango fruit: characterization, associated antioxidant capacity and application as a bakery product ingredient. LWT – Food Sci Technol. 2007;40(4):722–9.
6. Galanakis CM. Phenols recovered from olive mill wastewater as additives in meat products. Trends Food Sci Technol. 2018;79:98–105.
7. Zhang ZH, Wang LH, Zeng XA, Han Z, Brennan CS. Non-thermal technologies and its current and future application in the food industry: a review. Int J Food Sci Technol. 2019;54:1–13.
8. Huarte E, Juániz I, Cid C, de Peña MP. Impact of blanching and frying heating rate/time on the antioxidant capacity and (poly)phenols of cardoon stalks (Cynara cardunculus L. var. altilis DC). Int J Gastron Food Sci. 2021;26:100415.
9. Ordóñez-Santos LE, Esparza-Estrada J, Vanegas-Mahecha P. Ultrasound-assisted extraction of total carotenoids from mandarin epicarp and application as natural colorant in bakery products. LWT. 2021;139:110598.
10. Gabrić D, Barba F, Roohinejad S, Gharibzahedi SMT, Radojčin M, Putnik P, et al. Pulsed electric fields as an alternative to thermal processing for preservation of nutritive and physicochemical properties of beverages: A review. J Food Process Eng. 2018;41(1):e12638.
11. Li W, Pickard MD, Beta T. Effect of thermal processing on antioxidant properties of purple wheat bran. Food Chem. 2007;104(3):1080–6.
12. Li R, Kou X, Cheng T, Zheng A, Wang S. Verification of radio frequency pasteurization process for in-shell almonds. J Food Eng. 2017;192:103–10.
13. Li R, Kou X, Zhang L, Wang S. Inactivation kinetics of food-borne pathogens subjected to thermal treatments: a review. Int J Hyperth. 2018;34:177–88.
14. Songtipya P, Sengsuk T, Songtipya L, Prodpran T, Kalkornsurapranee E. A novel natural rubber pressure sensitive adhesive patch amended with cinnamon oil for preserving bakery product. Food Packag Shelf Life. 2021;29:100729.
15. Bangar SP, Chaudhary V, Thakur N, Kajla P, Kumar M, Trif M. Natural antimicrobials as additives for edible food packaging applications: a review. Foods. 2021;10:2282.
16. Taghvaei M, Jafari SM. Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. J Food Sci Technol. 2015;52:1272–82.
17. Rodríguez Sauceda EN. Uso de agentes antimicrobianos naturales en la conservación de frutas y hortalizas. Ra Ximhai. 2011;7(1):153–70.
18. Celli GB, Brooks MSL. Impact of extraction and processing conditions on betalains and comparison of properties with anthocyanins — a current review. Food Res Int [Internet]. 2017;100:501–9. Available from: https://www.sciencedirect.com/science/article/pii/S0963996916303623.
19. Herbach K, Stintzing F, Carle R. Betalain stability and degradation—structural and chromatic aspects. J Food Sci. 2006;71:R41–50.
20. Mikołajczyk-Bator K, Czapski J. Effect of pH changes on antioxidant capacity and the content of betalain pigments during the heating of a solution of red beet betalains. Pol J Food Nutr Sci. De Gruyter Open. 2017;67:123–8.

21. Gengatharan A, Dykes GA, Choo WS. Stability of betacyanin from red pitahaya (Hylocereus polyrhizus) and its potential application as a natural colourant in milk. Int J Food Sci Technol. 2016;51(2):427–34.
22. Kumorkiewicz A, Wybraniec S. Thermal degradation of major gomphrenin pigments in the fruit juice of Basella alba L. (Malabar Spinach). J Agric Food Chem. 2017;65(34):7500–8.
23. de Azeredo HMC. Nanocomposites for food packaging applications. Food Res Int [Internet]. 2009;42(9):1240–53. Available from: https://www.sciencedirect.com/science/article/pii/S096399690900091X.
24. Gonçalves LCP, Tonelli RR, Bagnaresi P, Mortara RA, Ferreira AG, Bastos EL. A nature-inspired betalainic probe for live-cell imaging of Plasmodium-infected erythrocytes. PLoS One. 2013;8(1):e53874.
25. Tupuna DS, Paese K, Guterres SS, Jablonski A, Flôres SH, Rios A d O. Encapsulation efficiency and thermal stability of norbixin microencapsulated by spray-drying using different combinations of wall materials. Ind Crop Prod. 2018;111:846–55.
26. Bamidele OP, Emmambux MN. Encapsulation of bioactive compounds by "extrusion" technologies: a review. Crit Rev Food Sci Nutr. 2021;61:3100–18.
27. Katouzian I, Jafari SM. Nano-encapsulation as a promising approach for targeted delivery and controlled release of vitamins. Trends Food Sci Technol. 2016;53:34–48.
28. Jafari SM, Assadpoor E, He Y, Bhandari B. Encapsulation efficiency of food flavours and oils during spray drying. Dry Technol. 2008;26:816–35.
29. Liao W, Badri W, Dumas E, Ghnimi S, Elaissari A, Saurel R, et al. Nanoencapsulation of essential oils as natural food antimicrobial agents: an overview. Appl Sci (Switzerland). 2021;11:5778.
30. Hamid SS, Wakayama M, Ashino Y, Kadowaki R, Soga T, Tomita M. Effect of blanching on the concentration of metabolites in two parts of Undaria pinnatifida, Wakame (leaf) and Mekabu (sporophyll). Algal Res. 2020;47:101829.
31. Sicari V, Loizzo MR, Silva AS, Romeo R, Spampinato G, Tundis R, et al. The effect of blanching on phytochemical content and bioactivity of hypochaeris and hyoseris species (Asteraceae), vegetables traditionally used in Southern Italy. Foods. 2021;10(1):32.
32. Otálora CM, Bonifazi EL, Fissore EN, Basanta MF, Gerschenson LN. Thermal stability of betalains in by-products of the blanching and cutting of Beta vulgaris L. Var conditiva. Pol J Food Nutr Sci. 2020;70(1):15–24.
33. Xiao HW, Pan Z, Deng LZ, El-Mashad HM, Yang XH, Mujumdar AS, et al. Recent developments and trends in thermal blanching – a comprehensive review. Inf Process Agric. 2017;4:101–27.
34. Ruiz V, Alonso R, Salvador M, Condón S, Condón-Abanto S. Impact of shoulders on the calculus of heat sterilization treatments with different bacterial spores. Food Microbiol. 2021;94:103663.
35. Deshaware S, Gupta S, Singhal R, Variyar PS. Influence of different pasteurization techniques on antidiabetic, antioxidant and sensory quality of debittered bitter gourd juice during storage. Food Chem. 2019;285:156–62.
36. Cheng C x, Jia M, Gui Y, Ma Y. Comparison of the effects of novel processing technologies and conventional thermal pasteurisation on the nutritional quality and aroma of Mandarin (Citrus unshiu) juice. Innov Food Sci Emerg Technol. 2020;64:102425.
37. Hradecky J, Kludska E, Belkova B, Wagner M, Hajslova J. Ohmic heating: a promising technology to reduce furan formation in sterilized vegetable and vegetable/meat baby foods. Innov Food Sci Emerg Technol. 2017;43:1–6.
38. Giraldo Gil A, Ochoa González OA, Cardona Sepúlveda LF, Alvarado Torres PN. Venting stage experimental study of food sterilization process in a vertical retort using temperature distribution tests and energy balances. Case Stud Therm Eng. 2020;22:100736.
39. Martín-Vertedor D, Rodrigues N, Marx ÍMG, Veloso ACA, Peres AM, Pereira JA. Impact of thermal sterilization on the physicochemical-sensory characteristics of Californian-style black olives and its assessment using an electronic tongue. Food Control. 2020;117:107369.

40. Triana A, Maruddin F, Malaka R. Supplementation of matoa (Pometia pinnata) leaf extract and alginate suppressed the growth of Staphylococcus aureus and Escherichia coli in pasteurized milk. In: IOP conference series: earth and environmental science. IOP Publishing; 2020.
41. Poudel A, Gachumi G, Wasan KM, Bashi ZD, el Aneed A, Badea I. Development and characterization of liposomal formulations containing phytosterols extracted from canola oil deodorizer distillate along with tocopherols as food additives. Pharmaceutics. 2019;11(4):185.
42. Noriega D, Villavicencio C, Domínguez L, Avilés R, Echavarría AP. Determinación del valor nutricional y la inocuidad de un puré infantil usando aditivos naturales. Ing Ind Actual Nuevas Tend [Internet]. 2019;VI(23):57–74. Available from: https://www.redalyc.org/articulo.oa?id=215067134005.
43. Ahmed J, Ramaswamy HS. Microwave pasteurization and sterilization of foods. In: Shafiur Rahman M, editor. Handbook of food preservation. CRC Press; 2020.
44. Leiton-Ramírez YM, Ayala-Aponte A, Ochoa-Martínez CI. Physicochemical properties of guava snacks as affected by drying technology. Processes. 2020;8(1):106.
45. Padilla-Frías KA, Granados-Conde C, Leon-Mendez G, Pineda YA, Torrenegra-Alarcon M. Evaluación de la influencia de la temperatura en procesos de secado. @ limentech. Cienc Tecnol Aliment. 2020;16(1):98–110.
46. Correâ-Filho LC, Lourenço MM, Moldaõ-Martins M, Alves VD. Microencapsulation of β-carotene by spray drying: effect of wall material concentration and drying inlet temperature. Int J Food Sci. 2019;2019:8914852.
47. Guiné R. The drying of foods and its effect on the physical-chemical sensorial and nutritional properties. Int J Food Eng. 2018;24:4.
48. Dantas D, Pasquali MA, Cavalcanti-Mata M, Duarte ME, Lisboa HM. Influence of spray drying conditions on the properties of avocado powder drink. Food Chem. 2018;266:284–91.
49. Chiou A, Kalogeropoulos N. Virgin olive oil as frying oil. Compr Rev Food Sci Food Saf. 2017;16(4):632–46.
50. Alves G, de Carvalho A, Olmo-García L, Rachel Antunes Gaspar B, Carrasco-Pancorbo A, Naciuk Castelo-Branco V, Guedes TA. Evolution of the metabolic profile of virgin olive oil during deep-frying: assessing the transfer of bioactive compounds to the fried food. Food Chem [Internet]. 2022;380:132205. Available from: https://www.sciencedirect.com/science/article/pii/S0308814622001662.
51. Wang Y, Wu X, McClements DJ, Chen L, Miao M, Jin Z. Effect of new frying technology on starchy food quality. Foods. 2021;10(8):1852.
52. Sordini B, Veneziani G, Servili M, Esposto S, Selvaggini R, Lorefice A, et al. A quanti-qualitative study of a phenolic extract as a natural antioxidant in the frying processes. Food Chem. 2019;279:426–34.
53. Shang A, Luo M, Gan RY, Xu XY, Xia Y, Guo H, et al. Effects of microwave-assisted extraction conditions on antioxidant capacity of sweet tea (Lithocarpus polystachyus rehd.). Antioxidants. 2020;9(8):678.
54. Perla V, Holm DG, Jayanty SS. Effects of cooking methods on polyphenols, pigments and antioxidant activity in potato tubers. LWT – Food Sci Technol. 2012;45(2):161–71.
55. Khan MK, Ahmad K, Hassan S, Imran M, Ahmad N, Xu C. Effect of novel technologies on polyphenols during food processing. Innov Food Sci Emerg Technol. 2018;45:361–81.
56. Hayat K, Zhang X, Farooq U, Abbas S, Xia S, Jia C, et al. Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. Food Chem. 2010;123(2):423–9.
57. Kumar K, Srivastav S, Sharanagat VS. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: a review. Ultrason Sonochem. 2021;70:105325.
58. Nguyen TP, Songsermpong S. Microwave processing technology for food safety and quality: A review. Agric Nat Resour. 2022;56(1):57–72.
59. Icier F. Ohmic heating of fluid foods. In: Cullen PJ, Tiwari BK, Valdramidis VP, editors. Novel thermal and non-thermal technologies for fluid foods. Academic Press; 2012.

60. Guzik P, Kulawik P, Zając M, Migdał W. Microwave applications in the food industry: an overview of recent developments. Crit Rev Food Sci Nutr. 2021:1–20. https://doi.org/10.1080/10408398.2021.1922871.
61. el Darra N, Grimi N, Vorobiev E, Louka N, Maroun R. Extraction of polyphenols from red grape pomace assisted by pulsed ohmic heating. Food Bioprocess Technol. 2013;6(5):1281–9.
62. Guida V, Ferrari G, Pataro G, Chambery A, di Maro A, Parente A. The effects of ohmic and conventional blanching on the nutritional, bioactive compounds and quality parameters of artichoke heads. LWT – Food Sci Technol. 2013;53(2):569–79.

Chapter 10
Effect of Nonthermal Treatments on the Properties of Natural Food Additives

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10.1 Introduction

Consumers demand fresh and minimally processed foods with natural ingredients that enhance health or prevent disease. This trend raises industries' and researchers' interest in developing processing techniques that result in higher quality foods free of chemical additives. Thermal treatment, commonly used to increase the shelf life of foods through the inactivation of microorganisms and enzymes, has detrimental effects on processed foods'nutritional and sensory attributes, including the loss of antioxidant activity, phenolics, and discoloration. Nonthermal technologies have been highly recommended in the food industry as an alternative to conventional processes to prevent quality losses in food products. High-pressure processing (HHP), ultrasound (Us), pulsed light (PL), UV-light, cold plasma (CP), pulsed electric field (PEF), and radio frequency (RF) are some nonthermal techniques of the emerging research that can improve, maintain or change properties of compounds related to natural additives in food manufacturing.

A suitable nonthermal technology may promote several modifications in natural food additives (NFAs), improving sensory and texture properties, digestibility, and antimicrobial and antioxidant activities. An increase efficiency when extracting intracellular compounds such as phenolics, pigments, starches, and proteins has been the main effect reported in using nonthermal technologies in foods [1–6]. In contrast, structural changes such as depolarization and crosslink are reported mainly in macromolecules (e.g., starch and protein) [7–9]. Furthermore, technologies such

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as CP and PEF can act as abiotic stressors, inducing reactions and the formation of bioactive compounds [4, 5, 10, 11].

In food processing, aiming at improving natural compounds, nonthermal technologies have advantages over traditional processes due to the possibility of reducing the use of solvents, energy efficiency, and shorter processing time, in addition to the quality of the final product [1]. Knowing the impact of these emerging technologies on changing the food compounds allows the development of strategies to improve food properties, reduce the number and amount of additives in a given product, or improve the quality of natural compounds that can be used as food additives. The following sections will present the impact of nonthermal techniques benefiting NFAs and discuss the mechanisms associated with them.

10.2 High-Pressure Processing

High-pressure processing (HPP) – also known as high isostatic pressure (HIP) or high hydrostatic pressure (HHP) – is a nonthermal treatment using pressures up to 1000 MPa into a product in controlled time and temperature conditions [12]. The observed HPP effects converges to increase surface hydrophobicity, change the structure of the non-covalent bonds, and cause molecules denaturation and aggregation (e.g., proteins) [13]. HPP can also improve protein functionality and digestibility of cereals and legumes [14] while reducing microorganisms for juice preservation [15].

High-pressure homogenization (HPH) – also called dynamic high-pressure (DHP) – imposes high-pressure conditions by pumping liquid food through a tiny gap in a valve, which results in a high velocity that causes high shear stresses. Consequently, it causes changes in food rheological properties [8]. HPH combines the effectiveness of high-frequency vibration, high-velocity impact, quick pressure drop, cavitation, and intense shear stress in a short time [16]. Typical HPH pressures are moderate and usually up to 100 MPa [17], while HPP can reach ten times more. HPH was also recently applied to food products aiming at microbial inactivation and changes in the protein's techno-functional properties [12].

High pressures favor extracting bioactive compounds from plants (e.g., carotenoids, chlorophylls, sterols, fibers, and phenolics) [8] by decreasing solvent consumption, increasing extraction yields, and shortening the extraction time. Studies demonstrated that HPP could be applied to foods and increase their antioxidant capacity by maintaining/improving the concentration of anthocyanins [18, 19], ascorbic acid/vitamin C [20], tocopherols/vitamin E [21], which are natural antioxidants in foods. Likewise, the carotenoids (lutein, α-carotene, and β-carotene), which can be used as colorings and antioxidants, were evaluated after HPP treatment (600 MPa) and no differences between the untreated and treated pumpkin purées was observed [22]. Table 10.1 shows more examples of high-pressure technology applied to foods aiming their role as natural additives.

Table 10.1 Examples of high-pressure processing (HPP) applied to foods aiming their role as natural additives

Food	Natural additive present	Additive role	HPP conditions	Results	Reference
Apple juice	Ascorbic acid, quercetin, gallic acid, procyanidin B2, and catechin	Antioxidant	300–600 MPa 5–15 min	HPP treatment caused the degradation of ascorbic acid, quercetin, gallic acid, procyanidin B2, and catechin after storage	[15]
Persimmon fruit	Gallic acid and catechin	Antioxidant	100 MPa 10 min	Low intensity of HPP significantly increased the extractability of phenolics	[23]
Nectarine purée	Criptoxantin, β-carotene, zeaxanthin and lutein, and gallic acid	Coloring and antioxidant	450–600 MPa 5–10 min	HPP at 600 MPa/10 min showed the highest phenolics content. Zeaxanthin + lutein and criptoxantin was significantly highest in purées treated at the lowest pressure intensity and shortest holding time	[19]
Açaí juice	Anthocyanins, tocopherols, and gallic acid	Antioxidant	400–600 MPa 5 min 20 °C	HPP was effective for the preservation of anthocyanins and phenolics. Tocopherols activity were not affected	[21]
Turmeric	Gallic acid, curcumin, ferulic acid, vanillin, and vanillic acid	Antioxidant	100–550 MPa 15 min	HHP at 400 MPa for 20 min was the optimal extraction condition for the highest antioxidant activity	[24]
White tea	Catechin and caffeine	Antioxidant	300–500 MPa 120–600 s	The maximum total phenolic content (1949.2 mg/L) and total antioxidant activity (91.9%) were achieved at 300 MPa for 600 s	[25]
Coconut water	Ascorbic acid and gallic acid	Antioxidant	500 MPa 5 min	HPP treatment substantially delayed losses of ascorbic acid, phenols, and antioxidant capacity	[20]

(continued)

Table 10.1 (continued)

Food	Natural additive present	Additive role	HPP conditions	Results	Reference
Pomegranate juice	Anthocyanin and rutin	Antioxidant	400–600 MPa 3–10 min 20 °C	For anthocyanins and antioxidant activity, the maximal retention of 95.69% and 95.89% was achieved at 600 MPa/3 min	[18]
Jussara juice	Anthocyanin	Antioxidant	200–500 MPa 5–10 min	While 200 MPa/5 min retained anthocyanins, 82% were lost at the 500 MPa/10 min	[26]
Carambola purée	β-carotene, gallic acid, and rutin	Coloring and antioxidant	200–800 MPa 5–15 min 25 °C	The color change was caused by the β-carotene release. The phenolics and the antioxidant activity increased with the increase of pressure	[27]
Fruit juice mixture sweetened with *Stevia rebaudiana*	Ascorbic acid, anthocyanin, and gallic acid	Antioxidant	300–500 MPa 5–15 min	HPP conducted at 300 MPa/14 min led to a beverage with the greatest presence of antioxidant compounds	[28]
Citrus beverages	Hesperidin, anthocyanins (cyanidin 3-O- glucoside), and ascorbic acid	Antioxidant	450–600 MPa 180 s	Phenolic compounds were little affected by the HPP. Ascorbic acid showed significant degradation after processing under any condition	[29]
Soybean protein isolate	Protein	Foaming properties	100–300 MPa	Foaming increased after HPP treatment	[30]
Peanut protein isolate	Protein	Water- and oil-holding capacities	50–200 MPa 5 min	HPP can be used to modify the properties of peanut protein isolate at the appropriate pressure within a short time	[31]

(continued)

Table 10.1 (continued)

Food	Natural additive present	Additive role	HPP conditions	Results	Reference
Sweet potato protein	Protein	Water-holding and gelation properties	250–550 MPa pH 3–9	The hardness, springiness, chewiness, and water-holding capacity of gels treated at moderate pressure (250 and 400 MPa) were improved, leading to a compact and uniform three-dimensional gel network	[32]
Sweet potato protein	Protein	Gelation properties	400 MPa 25 °C 30 min	Textural properties of gels were improved by sulfur-containing amino acids and HHP	[13]
Fababean protein	Protein	Emulsifying and foaming properties	103–207 MPa 32–45 °C 6 cycles	Improvement in foaming capacity and decreased emulsifying capacity by HPP	[33]
Potato protein isolate	Protein	Gelation properties	300–500 MPa	300–500 MPa allows the formation of physical gels only at pH 3, and when the system crosses 30 °C by adiabatic heating during pressurization	[34]
Pork batters with gum	Protein-gum	Water-holding and gelation	400 MPa 15 min	Water-holding capacity and gel strength increased with the increase in pressure	[35]

10.3 Ultrasound

Ultrasound (Us) technique uses low-frequency and high-intensity soundwaves, ranging from 20 to 100 kHz [36]. As consequence, it leads to the cavitation phenomenon, forming gas bubbles within the liquid phase and causing local microexplosions and volume increase [37]. The Us provides high shear forces in the extractive agent, accelerates the mass transfer of bioactive compounds [38], and improves solubility due to cellular structure's high stress and deformation [37]. The increased temperature, turbulence, and cavitation caused by the Us treatment also increase extraction efficiency [8] and reduce extraction time and protein aggregates [39]. Additionally, the cavitation bubbles result in micro-jetting and particle breakdown, improve solvent permeation into the food matrix, and enhance protein functionality [36, 39].

High-intensity ultrasound is a quick and cost-effective technology to modify proteins' structural and functional properties [40] while recovering valuable bioactive compounds, such as natural additives from plants (e.g., carotenoids, chlorophylls, and phenolics) [41]. Table 10.2 displays examples of Us technology applied to foods aiming their role as natural additives.

10.4 Cold Plasma

Plasma is described as ionized gas containing reactive species (e.g., in air it forms oxygen reactive species, ROS: atomic oxygen (O), superoxide anion (O_2^-), ozone (O_3), singlet oxygen ($^1O_2$), and hydroxyl radical (OH•), and reactive nitrogen species, RNS: atomic nitrogen (N), nitric oxide (NO•), and nitric dioxide (NO_2•)), ultraviolet radiation (UV), free radicals, electrons, and charged particles [6, 8]. Usually, the plasma is generated by applying a high electrical potential difference between two electrodes that causes gas ionization due to free electrons colliding with the gas molecules. The plasma is classified as thermal and nonthermal. There is a local thermal equilibrium in thermal plasma, and all the species are at the same temperature. Conversely, in the nonthermal or cold plasma (CP), there is no local thermal equilibrium, characterized by an electron temperature much above that of the ions and neutral molecules [60].

CP technology has a great diversity of applications in various industry sectors. Specifically, agency regulators have not yet approved the CP application in food [6]. However, a wide range of studies demonstrates the application of CP for nutritional improvements. For example, for a natural food addictive present in food products, CP can be used to alter the physicochemical properties of starches and proteins, the bioactive content and properties, modulate aromas, and change the pigment's color. In addition, CP effectively inactivates microorganisms [61] and enzymes [62], enhances antioxidant activity [63], and degrades mycotoxin [64], pesticides [65], and allergenic [66]. Table 10.3 displays examples of CP technology applied to foods aiming their role as natural additives.

The design aspects of each CP generating system and operational parametric setup lead to different CP properties and, consequently, different food product properties after the treatment. Among others, the most impacting characteristics of a CP system are the source (piece of equipment design), feed gas, electrode material, and operating humidity, frequency, and voltage [6]. Therefore, plasma induces numerous reactions, and the synergistic contributions of them make plasma chemistry rather complex. Besides, multiple reaction pathways are plausible, including activating complex metabolic pathways in fruits and vegetables [10, 67], in special when CP interacts with foods matrix, that are complex and multicomponent systems. The interactions of reactive plasma particles with each food component lead to specific changes in chemical composition, generation of new products, and altering the component characteristics [68].

Table 10.2 Examples of ultrasound (Us) technology applied to foods aiming their role as natural food additives

Food	Natural food additive present	Additive role	Us conditions	Results	Reference
Maqui berries	Anthocyanin and gallic acid	Antioxidant	10–70 °C 30–70% amplitude	The optimal extraction time was 15 min for gallic acid, while 5 min for anthocyanins	[42]
Raspberry seed oil	Tocopherol	Antioxidant	250 W 0–70 °C 40 kHz 10–40 min	30 min and 50 °C were the best conditions to extract tocopherol (15.1 mg/g sample)	[43]
Lemon balm and peppermint leaves	Gallic acid, carotenoids, and chlorophylls	Antioxidant	35 kHz 140 W 5–30 min 28.2–56.4 °C	A significant increase in all studied bioactive compounds was found during 5–20 min extraction. The maximum of total chlorophylls and carotenoids were determined during 20 min of ultrasonic extraction	[41]
Green propolis	Gallic acid	Antioxidant	40 kHz 20 min 25 °C	Extracts were suitable to produce natural ingredients with antioxidant capacity aiming for food use	[44]
Red beet	Betalain	Coloring and antioxidant	165 W 0–100% 30 °C	Us resulted in higher betalains content at low temperature using less extraction time	[45]
Mung bean coat	Gallic acid, catechin, coumaric acid, vitexin, and isovitexin	Antioxidant	70 °C 46 min	Compared with conventional methods (maceration and Soxhlet), optimized US was much more efficient for extracting antioxidant ingredients	[46]

(continued)

Table 10.2 (continued)

Food	Natural food additive present	Additive role	Us conditions	Results	Reference
Blueberry pomace	Anthocyanin, gallic acid, and catechin	Antioxidant	64 W 35 kHz	Us under slightly basic pH conditions positively affected total phenolic content and antioxidant activity compared to acidic pH, but lowered the anthocyanin content	[47]
Green tea	Gallic acid, catechins, caffeine, epicatechin gallate, ellagic acid, and astragalin	Antioxidant	360 W 25–85 °C 0–35 min	The combined treatment of tannase and ultrasound markedly increased the antioxidant activity of the green tea extract	[48]
Olive and fig leaves	Gallic acid, catechin, and carotenoids	Antioxidant and antimicrobial	375 W 10 min	Results showed that Us extracted more carotenoids than conventional extraction while impacting on higher flavonoids (olive leaves) and total phenolics (fig leaves). Extracts presented the highest bacterial growth inhibition and showed the highest anti-inflammatory activity	[49]
Thyme and sage	Chlorophylls, carotenoids (β-carotene, lutein, zeaxanthin)	Coloring and antioxidant	60 °C 10.3 MPa 3 cycles/10 min	The extracted pigments were determined in the range of 73.8–127.6 mg/100 g	[2]
Soybean protein isolate	Protein	Emulsifying properties	200–600 W	Us pretreatment results in hydrolysates with improved emulsifying capability	[50]

(continued)

Table 10.2 (continued)

Food	Natural food additive present	Additive role	Us conditions	Results	Reference
Flaxseed gum	β-carotene	Coloring and antioxidant	500 W 22 kHz 50% amplitude 10–30 min	Us showed highest β-carotene extraction when compared to microwave or alkaline extractions	[51]
Cashew-apple coproduct	Gallic acid and quercetin	Antioxidant	150 W 25 kHz 30 °C	The optimized process provided a great yield of gallic acid (750 mg/100 g) and quercetin (479 mg/100 g)	[52]
Sweet potato and wild carrot	β-carotene	Antioxidant	Ni	The Us approach is the preferred method for extracting β-carotene from carrots, sweet potato, and marketed formulations	[53]
Kiwi peel	Catechin and quercetin-3-O-glucoside	Antioxidant	5–500 W 20 kHz 1–45 min	The sonication at 94.4 W for 14.8 min, using 68.4% ethanol, resulted in a maximum of 1.5 mg of flavonoids per g of extract	[54]
Soybean protein isolate	Protein	Gelation and water-holding properties	20 kHz 150–450 W	Under 300 W, the gel hardness reached a maximum of 998.9 g, with a water-binding capacity of 87%	[55]
Pea protein concentrate	Protein	Emulsifying properties	412.5–712.5 W 336–582 s	Emulsions were greatly improved	[56]
Pea protein isolate	Protein	Foaming properties	20 kHz 30–90% 30 min	Foaming ability increased from 145.6 to 200% and foaming stability from 58 to 73.3%	[40]

(continued)

Table 10.2 (continued)

Food	Natural food additive present	Additive role	Us conditions	Results	Reference
Soybean and rice protein isolates and pea protein concentrate	Protein	Oil- and water-holding properties	20 kHz 562.5–712.5 W 120–600 s	Properties are improved as the dispersibility of protein materials increases (712.5 W, 600 s)	[57]
Tamarind seed protein isolate	Protein	Emulsifying, foaming, oil-and water-holding properties	100–200 W 15–30 min	The functional properties were the highest when both time and intensity of treatment were high	[58]
Shell eggs	Protein	Foaming properties	200–450 W 2–5 min 24 °C	Us caused stability of foam and maintained both foaming properties/ whipping capacity due to avoiding changes in the pH during storage	[59]

Ni Not informed

The phenolic compounds, responsible for the natural antioxidant activity, antimicrobial activity, flavor, and color in fruits and vegetables, can be altered by CP treatment by different mechanisms. The UV radiation and reactive species active cell defense mechanisms, acting as an abiotic stressor and inducing the biosynthesis of the phenolic [63], cause structural tissue damage, enhancing the extractability of bioactive compounds from the vacuoles [69, 70]. CP promotes the tannins depolymerization by breaking covalent bonds and forming smaller phenolic molecules (e.g., tannins to gallic acid) [71]. Also, the chemical transformations in the volatile compound profile are obtained [72, 73]. However, degradation of the compounds may occur depending on the treatment conditions, such as treatment time and feed gas [74, 75].

In response to the abiotic stress caused by CP, there is a higher consumption of sugars as a source of energy for the biosynthesis of phenolic compounds [63]. In contrast, the increase in sugar content is related to the depolymerization of starch, sucrose, and oligosaccharides, forming glucose, fructose, and other small-chain sugars [76]. The depolarization can also form molecules of small-chain of starch and oligosaccharide [9, 77].

CP also results in alterations of starch's chemical, physical, and mechanical properties. Crosslinking induced by CP occurs due to the cleavage at the extremity of two polymeric starch chains (C–OH) and forming of a new C–O–C linkage [78], resulting in a decrease in the viscosity and retrogradation, which increases the stability of the paste at high temperatures and on cooling [79].

Table 10.3 Examples of cold plasma (CP) technology applied to foods aiming their role as natural additives

Food	Natural food additive present	Additive role	CP conditions	Results	Reference
Grape pomace	Anthocyanins, quercetin, gallic acid, protocatechuic acid, and stilbenes	Coloring and antioxidant	DBD 60 kV 60 Hz He 5–15 min	CP pretreatment disrupted the epidermal cell structures, increased the grape peels hydrophilicity, accelerated grape drying, and increased the yield of phenolic extracts (10.9–22.8%) and antioxidant capacity (16.7–34.7%)	[75]
Camu-camu pulp	Terpenoids and sesquiterpenoids	Flavor and aroma	Glow discharge 80 kV 50 kHz Air 0.3 bar 10–30 min 10–30 mL/min	Chemical transformations in the volatile compound profile varied with the operating conditions. The main change in aroma profile was in the woody, pine, and spicy notes, and in the flavor profile was in the woody, camphoraceous, and citrus notes	[73]
Apple cubes and apple juice	Sucrose	Sweetness	DBD: 20 kV, 50–900 Hz, air, 15 min Glow discharge: 80 kV, 50 kHz, synthetic air, 0.3 bar, 10–30 min, 10–30 mL/min	Glow discharge decreases sucrose content and increases glucose, fructose, and malic acid, increasing sweetness power up to 27%. DBD reduced the sucrose, glucose, and fructose content and increased malic acid content, reducing the sweetness power up to 44%	[76]

(continued)

Table 10.3 (continued)

Food	Natural food additive present	Additive role	CP conditions	Results	Reference
Pomegranate juice	Tannins, anthocyanins, procyanidins, phenolic acids, and flavonol glycosides	Antioxidant	Plasma jet 25 kHz Ar 3–7 min 0.75–1.25 L/min	Pasteurization and CP increased total phenolic content by 29.55% and 33.03%, respectively. Ellagitannins depolymerization by CP increased ellagic acid content three times	[71]
Apple juice	Ascorbic acid, polyphenols, and pectin	Antioxidant	Plasma jet (spark and glow) 7.9–10.9 kV 20–65 kHz 1–5 min	Spark discharge at 10.5 kV for 5 min almost completely inactivated the polyphenol oxidase. As a result, juice color was lighter, increased antioxidant capacity (up to 17%), and polyphenols content (up to 69%), and the juice was stable during storage	[69]
Fresh-cut pitaya	Glucose and fructose gallic acid, protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, and p-coumaric acid	Sweetness, antioxidant, antimicrobial	DBD 60 kV Air 5 min	Treatment inhibited up to 2 log cfu growth of total aerobic bacteria, increased up to 27% phenolic total, and increased up to 21% antioxidant activity. The sugar consumption was triggered (after 48 h, glucose decreased 21.6% (control) and 27.4% (CP), fructose decreased by 20.1% (control) and 26.7% (CP)), increased energy supply and ROS signal, activating phenylpropanoid metabolism	[63]

(continued)

Table 10.3 (continued)

Food	Natural food additive present	Additive role	CP conditions	Results	Reference
Fresh-cut apples	Phenolics (Catechin, Epicatechin, Procyanidin, Caffeoylquinic acid, Quercetin)	Antioxidant	DBD 12.7 kHz 150 W Air 10–30 min	CP decreased the pH and the browning. Phenolic increase up to 20% at 10 min treatment and a progressive decrease was observed with increasing exposure time	[74]
Rice starch	Starch	Thickening and gelling	DBD 13.56 MHz 40–60 W Air 0.15 mbar 5–10 min	Treatment increased in gel hydration properties, syneresis, and final viscosities, decreased amylose content (from 29.3 to 22.8), pH (from 7.42 to 6.94), turbidity (from 30.0 to 18.5), gelatinization temperature, and pasting temperature. The addition of carboxyl and hydroxyl groups, the formation of fissures on granules, and depolymerization were observed	[80]

(continued)

Table 10.3 (continued)

Food	Natural food additive present	Additive role	CP conditions	Results	Reference
Potato starch	Starch	Thickening and gelling	Glow plasma 1.1 A 245 V N_2 and He 20 mbar 30–60 min	Polymerization and crosslink on molecular-scale increased starch branched, decreasing the viscosity and retrogradation, increasing the high-temperature paste stability and paste cooling stability. Gelatinization and pasting were facilitated, and the granules' surface etching was observed	[79]
Corn starches	Starch	Thickening and gelling	DBD and RF 13.56 MHz 90 W HMDSO 0.35 m^3/min 10 min	RF treatment formed cavities allowing the active species to modify the internal structure of the granule, increasing the amylose helix order and thermal stability. DBD treatment promoted a thicker coating deposition and HMDSO functional groups inclusion, increased the granular interaction and the decomposition temperature	[89]

(continued)

Table 10.3 (continued)

Food	Natural food additive present	Additive role	CP conditions	Results	Reference
Whey protein	protein	Emulsifier, gelling, flavor, and texture	DBD 70 kV Air 1–60 min	ROS and RNS increased yellow color and decreased pH. The treatment within 15 min caused mild oxidation, increased the carbonyl groups and the surface hydrophobicity, reduced free SH groups, improved foaming, and emulsifying capacity. Treatments at 30 and 60 min decreased the foaming and emulsifying capacity drastically and increased the foam stability	[87]
Wheat flour	Protein	Emulsifier, gelling, and texture	DBD 15–20 V 9 kHz Air 1–2 min	The treatment did not change the total aerobic bacterial count, mold count, concentration of non-starch lipids, non-polar, and glycolipids. At 20 V accelerated lipid oxidation, reducing total free fatty acids and phospholipids, and increased molecular protein weight, resulting in the stronger dough	[88]

The etching caused by CP treatment on the starch granule surface indicates surface-structural disorganization, easing water permeation into the granules, decreasing long-range crystallites and short-range orders, gelatinization temperature, and melting enthalpy [79]. The starch granules are oxidized by CP reactive oxygen species, decreasing the pH due to forming chemical groups with acidic characters, such as the carbonyl group [80].

Concerning natural pigments, generally phenolics, the main effect caused by CP treatment is their extraction from the vacuoles by the cell membrane degradation.

Also, the anthocyanin chromophores (responsible for coloring) can undergo oxidative cleavage and conjugated double bonds break by the reactive species, resulting in product color loss [81]. Besides, anthocyanins show different conformations at different pH, and as the CP treatment tends to decrease the medium pH, a color change can occur. Whereas chlorophyll degradation can occur by several routes, resulting in different colors. Colored intermediate compounds formed in the porphyrin ring's pathways remain unchanged. The additional oxidation cleaves these intermediates' porphyrin ring, producing fluorescent catabolites and, subsequently, colorless compounds [82, 83]. Groups can be changed or removed from the chlorophyll molecule periphery. This pathway can remove the phytol, forming green derivatives [83]. The acid environment also can change the chlorophyll color, where two hydrogen ions replace the Mg-atom of the porphyrin ring, converting chlorophylls into pheophytins, an olive-brown pigmentation [84, 85].

The main changes triggered by CP in proteins are ROS and RNS. The reactive species interact with the side chain of amino acid residue and protein polypeptide backbone, resulting in unfolding, crosslinking, fragmentation, and conformational changes [86]. The protein oxidation changes its functionalities, increasing the emulsifying and foaming capacity and foam stability at CP long exposure [87]. The CP in wheat flour promotes polymerization, solubility alteration, and forming of a gluten network, resulting in a stronger dough [88]. Also, CP can inactivate enzymes involved in undesirable reactions, such as peroxidase, polyphenol oxidase, and lipoxygenase, as well as the inactivation of the allergenic protein [6].

10.5 Pulsed Electric Field

The pulsed electric field (PEF) applies high voltage pulses for a very short time (from several nanoseconds to milliseconds) to a food product placed between two electrodes [8]. PEF induces the formation of irreversible or reversible pores in biological cell membranes, known as the electroporation phenomenon (cell electrical breakdown) [90, 91]. This nonthermal technology can improve food products through microbial inactivation due to the dielectric breakdown of the cell membrane and enzyme inactivation. The PEF pretreatment of food products is effective for osmotic dehydration [92], improvement of freezing and thawing processes [93], and reducing drying process time [94]. The main effect of PEF on bioactive compounds relays on the disruption of cells which increases the mass transfer of intracellular compounds, making them more available. As a result, bioactive extraction efficiency increases, shorting extraction time, reducing solvent consumption, and maintaining the quality of these compounds [5]. Table 10.4 displays examples of PEF technology applied to foods aiming their role as NFAs.

The PEF treatment efficacy depends, among other factors, on the electric field intensity, temperature, treatment time, pulse wave, and physical properties of food, such as electrical conductivity, size, and shape of cells. For example, foods with more current-conducting compounds within their cells (e.g., ions of dissociated

Table 10.4 Examples of pulsed electric field (PEF) technology applied to foods aiming their role as natural food additives

Food	Natural additive present	Additive role	PEF conditions	Results	Reference
Carrot	Phenolics	Antioxidant and pigment	0.8–3.5 kV/cm 5–30 pulses	At 5 pulses of 3.5 kV/cm and 30 pulses of 0.8 kV/cm was the highest increase in phenolics (about 40%) after storage for 24 h at 4 °C; color and hardness were maintained. Weight loss reached 9.3% at 3.5 kV/cm (the control was 1%). A higher increase in media conductivity was observed after treatment at 2 and 3.5 kV/cm. The cell viability was reduced up to 73% for carrots treated	[97]
Spinach	Chlorophyll carotenoids	Antioxidant and pigment	3.3–26.7 kV/cm 1 kHz 20 μs	PEF treatment increased the chlorophyll a, b, carotenoids, and antioxidant activity up to 26.3, 21.0, 41.5, 14%, respectively, compared to the untreated. In addition, promoted the crosslink reaction with other chlorophyll molecules and affected carotenoids' unsaturated bond, changing conformation from cis to trans	[101]
Tomato peel	Lycopene	Antioxidant and pigment	1–5 kV/cm 10 Hz 20 μs 10–833 pulse number	PEF before the solvent extraction process enhanced the extraction rate (27–37%), lycopene yields (12–18%), and antioxidant power (18%). PEF induced size reduction and separation between the plant cells due to pore formation and leakage of intracellular matter	[110]

(continued)

Table 10.4 (continued)

Food	Natural additive present	Additive role	PEF conditions	Results	Reference
Freshwater mussel	Protein	Nutrient	10–35 kV/cm 2 μs 2–12 pulse number 40–3000 Hz	The protein extraction yield was 77.08% at 20 kV/cm, 8 pulse number, and 2 h enzymolysis time. Compared with other extraction methods (NaCl, alkali, and enzyme method), the PEF increased the speed and yield extraction from the mussel	[103]
Canola seeds	Protein	Emulsifier, foaming, and water-holding	10–35 kV 100–1000 Hz 1–10 μs 60–210 s (residence time)	PEF pretreatment increased the functional properties of canola protein: solubility (up to 46%), water-holding capacity (up to 68%), emulsibility (up to 13%), emulsion stability (up to 21%), oil-holding capacity (up to 74%), foamability (up to 40%), and foam stability (up to 51%). PEF changed the secondary structure, increased free sulfhydryl groups, and surface hydrophobicity, and formed protein aggregates with low molecular mass	[107]
Macroalgae (*Ulva Ohnoi*)	Protein and starch	Nutrient	1 kV/cm 30 Hz 50 μs	PEF treatment increased the conductivity sample (indicating that treatment affected membrane permeability), and the extraction of starch, protein, and ash (60, 15, and 68%, respectively) compared to the control (52, 3, and 47%, respectively)	[104]

(continued)

Table 10.4 (continued)

Food	Natural additive present	Additive role	PEF conditions	Results	Reference
Esterified Potato starch	Starch	Emulsifier and digestibility	1.25–5 kV/cm 1000 Hz 40 μs 60 min (residence time)	The slowly digestible starch fractions increased from 6.6% (control) to 17.5% (PEF-treated). As the electric intensity was increased, more deformations, protrusions, and pits were observed on the starch granules' surface. The sample treated with higher electric intensity resulted in a stable emulsion	[109]
Waxy rice starch	Starch	Thickening and digestibility	3–50 kV/cm 1 kHz 40 μs	PEF treatment decreased the gelatinization temperature (up to 17%), enthalpy (up to 45%), crystallinity (up to 23%), and slowly digestible starch level (up to 23%), and increased rapidly digestible starch (up to 55%). The changes were more pronounced as the intensity of the electric field was increased	[108]

salts and charged molecules of proteins) are more susceptible to electroporation [95].

The PEF treatment increased the content of phenolic compounds and antioxidant activity of fruits and vegetables and improved the extraction of natural pigments (e.g., carotenoids and anthocyanins) [96, 97]. However, depending on the PEF operating parameters, it may decrease the bioactive compounds content [11, 95]. Also, different PEF parameters can modulate the chemical composition of bioactive extracts [98]. The co-pigmentation and pigments formation may be favored by PEF pretreatment, as observed for winemaking before the macerating fermentation step, in which the polyphenols extraction increased 48%, and the wine color attributes increased 56% [99].

The PEF voltage can cause dissociation of water and other molecules, producing free radicals and hydrogen peroxide [100]. Thus, PEF treatment can act as an abiotic stressor for the biosynthesis of secondary metabolites, increasing the phenolic content in the food products [97]. Also, the pigments chlorophyll are affected by free radicals, mainly the chemical bonds between the pyrrole ring and central magnesium ions of the molecule that can form the chlorophyll aggregated structures and increase the stability [101].

PEF treatment can influence biomacromolecules' physicochemical and functional properties [102]. In addition to improving the extraction of proteins [103] and starch [104] from the cell tissue, treating proteins with PEF can induce structural and functional changes. The ionization of various chemical groups or breaking of electrostatic interactions alters the secondary and tertiary structure of proteins, consecutively in the loss of α-helix and β-sheet, resulting in modifications such as unfolding, crosslinking, and aggregation of proteins [105–107]. Also, PEF alters starch properties by disintegrating amylopectin linkages and damaging starch granules, allowing water molecules to ingress into the crystalline region, decreasing crystallinity, gelatinization temperatures, and increasing water holding capacity [108]. These damages facilitate enzymes attack to the granules, increasing the digestibility. On the other hand, starch acetylation can increase the content of slowly digestible starch fractions [109].

10.6 Pulsed Light/UV-Light

Ultraviolet (UV) and pulsed light (PL) treatments are used as alternatives to chemical and thermal processing to inactivate microorganisms on surfaces, liquid foods, beverages, ingredients, and packaging, producing foods with better quality, extended shelf-life, and often with enhanced health benefits [111–113]. However, both technologies are based on irradiation, so the products can suffer photoreactions, depending on the food's optical properties, such as absorption, transmission, reflection, and the light spectrum emissions and irradiation doses. Once foodstuffs are exposed for too long and high doses of light energy, secondary products can be produced and cause matter changes, such as discoloration, off-flavors, loss of vitamins, and other essential nutrients [114–116].

The efficacy of both treatments is generally related to the absorption of UV-C light by microbial nucleic acids, causing photochemical changes, but for PL treatment, photothermal and photophysical changes on microorganisms are also related [111]. Therefore, UV-light technology is often found as monochromatic light in the UV-C spectrum ($\lambda = 254$ nm). UV-C devices work with low power; thus, long times are needed to be effective against microorganisms, which can cause the degradation of some other compounds, such as carotenoids, chlorophylls, flavonoids, and lipids [114]. In contrast, PL is found as a polychromatic light that includes ultraviolet (200–400 nm), visible light (380–780 nm), and infrared radiation (700–1100 nm) [111]. A capacitor stores high-intensity power energy and is released in short-intense pulses no longer than 2 ms. US Food and Drug Administration (FDA) approved PL to treat food surfaces with fluence levels not higher than 12 J cm^{-2} [117]. Together with other technologies and even mild temperatures, these technologies can enhance results further.

The literature is scarce on NFAs as ingredients in foodstuffs treated by light. However, natural compounds extracted from raw foods can be added to improve nutritional or functional properties of other products, Table 10.5. Plants exposed to

Table 10.5 Examples of UV and Pulse Light (PL) technology applied to foods aiming their role as natural food additives

Food	Natural food additive present	Additive role	Processing conditions	Results	Reference
Ergosterol incorporated into purple sweet potato pastes	Ergosterol transformed into Vitamin D2	Supplementation - Phosphorus and Calcium absorption	UVC-irradiation	Pastes with ergosterol concentrations lower than 0.65 mg/g could be well printed. Reducing the internal filling ratio was noted as an effective means to improve the conversion. When such a ratio was 70%, more formation of vitamin D2 at minimal use of raw materials could be realized	[120]
Mangoes	Vitamins C, B1, B3, B5, and B6	Participants in human metabolism	PL (3.6–10.8 J/cm^2)	Mangoes were pretreated by PL and then convective dried. Highest concentrations of vitamin C, B1, B3, and B5 were found in dried mangoes subjected to fluences PL treatment, while vitamin B6 decreased by 40 to 50% in the pretreated mangoes	[115]
Strawberry	Ascorbic acid (Vit C) and anthocyanin	Antioxidants	PL (4, 8, 12, and 16 J/cm^2)	Vitamin C and total anthocyanin contents of the samples treated at low energy doses were maintained, whereas those of slices treated at the highest energy dose decreased between 20 and 30%	[121]
Mushrooms	Gallic acid (GA), caffeic acid, chlorogenic acid, and quercetin contents	Antioxidants	PL (68.8 mJ/cm^2)	Short PL treatment (3 pulses each side) enhanced GA, caffeic acid, and quercetin contents of shiitake mushrooms	[129]

(continued)

Table 10.5 (continued)

Food	Natural food additive present	Additive role	Processing conditions	Results	Reference
White 'Gijnlim' asparagus	Anthocyanins	Antioxidant and colorant	After harvest, spears were exposed to weak white, red and blue light (30 μmol/m^2s) for 3 h and to UV-C (254 nm, 1 kJ/m^2) for 8 min	White-light triggered anthocyanin synthesis via an associated phenylalanine ammonia-lyase increase. Red light and UV-C irradiation tendentiously resulted in an anthocyanin inhibition or even degradation, coinciding with changes in phenylalanine ammonia-lyase activity	[116]
Sausage coated by curcumin-hydrogels	Curcumin	Photosensitizer, antioxidant, antimicrobial, and colorant	UV-A lamps (320–400 nm; 32 ± 0.2 W/m^2 for 5 and 15 min)	Curcumin can generate reactive oxygen species (ROS) in solution after excitation by a light causing microbial inactivation	[119]
Corn and potato	Starch	Thickener	UVB-irradiation	The potato amylose was more susceptible to changes upon UV-B irradiation, whereas corn ones. Similarly, functional properties were not significantly influenced by UV-B treatment	[130]
Cheese	Proteins	Nutrition	PL (1.3, 3.1, 7.5, 15 J/cm^2)	PL induced the formation of aggregates of small protein particles with lipids and carbohydrates, which reduced protein solubility. The formation of melanoidins and carbonyls confirmed protein photoreaction	[125]
Whey Protein Isolate (WPI) solution	Proteins	Nutrition/ Supplementation	PL (4–16 J/cm^2)	PL treatments increased the concentration of total and free sulfhydryl groups and protein carbonyls. In addition, PL treatments induced dissociation and partial unfolding of WPI, improving solubility and foaming ability	[127]

Egg white	Proteins	Functional properties	PL (1.75–31.5 J/cm^2)	PL caused browning, protein aggregation by disulfide exchange, and protein backbone cleavage. These structural modifications cause an increase in immunoreactivity and a decrease in gelling temperature. Also, foams treated by PL showed higher stability due to the jamming of protein aggregates and fragments in the fluid interstices between bubbles	[128]
Milk	β-Lactoglobulin and α-lactalbumin	Nutrition/ Supplementation	PL (4 cm from the lamp); 2.2 J/cm^2 per pulse (1, 3, 5, 7, and 10 pulses)	PL treatments showed no conformational changes in milk proteins despite aggregation by disulfide bonds, and the products had no oxidation appearance	[126]
Beeswax	Esters, hydrocarbons, fatty acids, and alcohols.	Glazing or coating for fruits and candies	UV light exposure (4.5 mW/cm^2 for 50 h)	The lifetime of beeswax at 23 °C was shortened by about 60%. FTIR showed the extinction of ester groups accompanied by an increase in free fatty acids content was observed	[131]
Fish	Lipids and proteins	Nutrition	Systematic review comparing UV-treatments with low doses (0.05–0.16 J/cm^2) and high (0.30–0.79 or about 0.30 J/cm^2).	Lipid and protein oxidation increased by 6% and 7% for low UV-light doses and 13% and 20% for high doses	[123]

light with different wavelengths have shown synthesized pigments, such as anthocyanins, associated with enzymes changes [116], which is often related to the visible blue light [118]. However, the exposition of these compounds to UV-C light causes their degradation [116]. Curcumin is natural pigment extracted from plants that have been discussed in the literature because of its photosensitizer property, which generates reactive oxygen species (ROS) after excitation, causing microbial inactivation [119].

Vitamins are crucial for human metabolism, and many foods can be fortified with these additives, which can be natural or synthetic. The literature reports UV-C light transforming ergosterol into vitamin D2 [120], and to break chemical bonds between vitamin B3 and nucleotides, and vitamin B5 and coenzyme A, turning into more bioavailable vitamins [115]. Nevertheless, light radiation also degrades vitamins C and B6 [115, 121].

Light control is essential in the flavor stability of vegetable oils and other unsaturated fats. The literature reports many cases of light-induced oxidation, as rapeseed, corn, soybean, and coconut oils and milk fat subjected to light in the wavelength ranging from 350 to 750 nm [122]. Fishes are rich in unsaturated fatty acids such as Omega-3 polyunsaturated fatty acids (PUFAs), a natural health additive used in supplements. Lipid oxidation increased by 6% when a sample was submitted to low UV-light doses and 13% for high doses [123]. A study on the addition of essential oils to foods aiming to improve the lethality of a UV-light treatment showed a synergistic effect on inactivating biofilms of *S.* Typhimurium [124].

Proteins are functional molecules that, after exposure to PL, aggregate with lipids and carbohydrates, reducing their solubility [125]. In milk proteins, it was reported that the only conformational modification was the aggregation of disulfide bonds [126]. However, when whey protein isolated (WPI) was exposed to PL treatment, its solubility and foaming ability were improved due to the dissociation and partial unfolding of WPI [127]. In addition, egg white proteins treated by PL showed structural changes, resulting in different functional properties, such as increased immunoreactivity, decreased gelling temperature, and higher foam stability [128].

UV-light and PL treatment have shown the potential to inactivate bacteria in clear and transparent liquids. However, their efficiency is compromised as turbidity increases, and for solid foods; low absorption and shadowing effects are challenging to be solved for the application of PL in the food industry. On the other hand, photodegradation products are restricted to the product's surface, often having low impact on the sensory and nutritional properties of the products.

10.7 Conclusions

The technologies reported herein are attractive due to their capacity to produce and preserve natural additives in foods, superior to the food quality when conventional thermal processes are applied for inactivating pathogenic and spoilage microorganisms and enzymes. In addition, those nonthermal methods cause structural

changes in cell membranes and structural modifications in some (macro)molecules, favoring extraction, digestibility, and desirable functional/nutritional properties.

Some of these emerging nonthermal technologies are on a small scale (laboratory or pilot level) and need to be scaled up before industrial use. On the other hand, the development of industrial equipment after scientific development has been intense and fast, as is the case of high-pressure systems. It is crucial to consider governmental regulations in each country and the safety aspects of each pair of technology-food product. Furthermore, costs, cultural changes, and consumer awareness are challenges in implementing a nonthermal process to obtain and modify natural compounds. Based on the state-of-the-art, these emerging nonthermal methods will keep evolving and reaching the food industry since they require fewer chemical additives, favoring natural additives usage.

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References

1. Barbhuiya RI, Singha P, Singh SK. A comprehensive review on impact of non-thermal processing on the structural changes of food components. Food Res Int [Internet]. 2021;149(April):110647. Available from: https://doi.org/10.1016/j.foodres.2021.110647.
2. Cvitkovi D, Lisica P, Repajic M, Pedisic S, Dragovic-Uzelac V, Balbino S. Composition and antioxidant properties of pigments of extraction methods. Foods. 2021;10(2477):1–16.
3. Abera G. Review on high-pressure processing of foods. Cogent Food Agric. 2019;5(1):1568725.
4. Saremnezhad S, Soltani M, Faraji A, Hayaloglu AA. Chemical changes of food constituents during cold plasma processing: a review. Food Res Int [Internet]. 2021;147(June):110552. Available from: https://doi.org/10.1016/j.foodres.2021.110552.
5. Barbosa-Cánovas GV, Donsì F, Yildiz S, Candoğan K, Pokhrel PR, Guadarrama-Lezama AY. Nonthermal processing technologies for stabilization and enhancement of bioactive compounds in foods [Internet]. Food Eng Rev. Springer US. 2021. Available from: https://doi.org/10.1007/s12393-021-09295-8.
6. Laroque DA, Seó ST, Valencia GA, Laurindo JB, Carciofi BAM. Cold plasma in food processing: design, mechanisms, and application. J Food Eng. 2022;312(April 2021):110748.
7. Chao D, Jung S, Aluko RE. Physicochemical and functional properties of high pressure-treated isolated pea protein. Innov Food Sci Emerg Technol. 2018;45:179–85.
8. Sá AGA, Laurindo JB, Moreno YMF, Carciofi BAM. Influence of emerging technologies on the utilization of plant proteins. Front Nutr. 2022;9(809058):1–18.
9. Thirumdas R, Kadam D, Annapure US. Cold plasma: an alternative technology for the starch modification. Food Biophys. 2017;12(1):129–39.
10. Fernandes FAN, Rodrigues S. Cold plasma processing on fruits and fruit juices: a review on the effects of plasma on nutritional quality. Processes. 2021;9(12):2098.

11. Gagneten M, Leiva G, Salvatori D, Schebor C, Olaiz N. Optimization of pulsed electric field treatment for the extraction of bioactive compounds from blackcurrant. Food Bioprocess Technol. 2019;12(7):1102–9.
12. Júnior BRCL, Tribst AAL, Cristianini M. Effect of high-pressure technologies on enzymes applied in food processing. In: Şentürk M, editor. Enzyme inhibitors and activators. London: IntechOpen; 2017. p. 49–72.
13. Zhao Z, Mu T, Zhang M, Richel A. Effects of sulfur-containing amino acids and high hydrostatic pressure on structure and gelation properties of sweet potato protein. Food Bioprocess Technol. 2019;12(11):1863–73.
14. Belmiro RH, Artigiani A, Tribst L, Cristianini M. Impact of high pressure in hydration and drying curves of common beans (Phaseolus vulgaris L.). Innov Food Sci Emerg Technol. 2018;28:279–85.
15. Szczepańska J, Pinto CA, Skąpska S, Saraiva JA, Marszałek K. Effect of static and multi-pulsed high pressure processing on the rheological properties, microbial and physicochemical quality, and antioxidant potential of apple juice during refrigerated storage. LWT – Food Sci Technol. 2021;150(June):112038.
16. Leite TS, Sastry SK, Cristianini M. Effect of concentration and consistency on ohmic heating. J Food Process Eng. 2018;41:1–9.
17. Kubo MTK, Augusto PED, Cristianini M. Effect of high pressure homogenization (HPH) on the physical stability of tomato juice. Food Res Int. 2013;51(1):170–9.
18. Ma Y, Yin X, Bi X, Su F, Liang Z, Luo M, et al. Physicochemical properties and bioactive compounds of fermented pomegranate juice as affected by high-pressure processing and thermal treatment. Int J Food Prop [Internet]. 2019;22(1):1250–69. Available from: https://doi.org/10.1080/10942912.2019.1640737.
19. García-Parra J, González-Cebrino F, Delgado J, Lozano M, Hernández T, Ramírez R. Effect of thermal and high-pressure processing on the nutritional value and quality attributes of a nectarine purée with industrial origin during the refrigerated storage. J Food Sci. 2011;76(4):618–25.
20. Ma Y, Xu L, Wang S, Xu Z, Liao X, Cheng Y. Comparison of the quality attributes of coconut waters by high-pressure processing and high-temperature short time during the refrigerated storage. Food Sci Nutr. 2019;7(4):1512–9.
21. Silveira TFF, Cristianini M, Kuhnle GG, Ribeiro AB, Filho JT, Godoy HT. Anthocyanins, non-anthocyanin phenolics, tocopherols and antioxidant capacity of açaí juice (Euterpe oleracea) as affected by high pressure processing and thermal pasteurization. Innov Food Sci Emerg Technol [Internet]. 2019;55(February):88–96. Available from: https://doi.org/10.1016/j.ifset.2019.05.001.
22. González-Cebrino F, Durán R, Delgado-Adámez J, Contador R, Bernabé RR. Impact of high pressure processing on color, bioactive compounds, polyphenol oxidase activity, and microbiological attributes of pumpkin purée. Food Sci Technol Int. 2016;22(3):235–45.
23. Ketnawa S, Hamanaka D, Wongpoomchai R, Ogawa Y, Setha S. Low intensity of high pressure processing increases extractable recovery of polyphenols and antioxidant activities of non-astringent persimmon fruit. LWT – Food Sci Technol [Internet]. 2021;151(February):112162. Available from: https://doi.org/10.1016/j.lwt.2021.112162.
24. Choi Y, Kim W, Lee J, Youn S, Lee H, Baik M. Enhanced antioxidant capacity of puffed turmeric (Curcuma longa L.) by high hydrostatic pressure extraction (HHPE) of bioactive compounds. Foods. 2020;9(11):1690.
25. Uzuner S, Evrendilek GA. Functional responses of cold brewed white tea to high pressure processing. J Food Process Eng. 2019;42(5):1–8.
26. Oliveira AA, Torres AG, Perrone D, Monteiro M. Effect of high hydrostatic pressure processing on the anthocyanins content, antioxidant activity, sensorial acceptance and stability of Jussara (Euterpe edulis) juice. Foods. 2021;10(10):2246.

27. Zhang S, Zheng C, Zeng Y, Zheng Z, Yao X, Zhao Y, et al. Mechanism of colour change of carambola puree by high pressure processing and its effect on flavour and physicochemical properties. Int J Food Sci Technol. 2021;56(11):5853–60.
28. Carbonell-Capella JM, Barba FJ, Esteve MJ, Frígola A. High pressure processing of fruit juice mixture sweetened with Stevia rebaudiana Bertoni: optimal retention of physical and nutritional quality. Innov Food Sci Emerg Technol [Internet]. 2013;18:48–56 . Available from: https://doi.org/10.1016/j.ifset.2013.01.011.
29. Salar FJ, Periago PM, Agulló V, García-Viguera C, Fernández PS. High hydrostatic pressure vs. thermal pasteurization: the effect on the bioactive compound profile of a citrus maqui beverage. Foods. 2021;10(10):2416.
30. Martínez KD, Ganesan V, Pilosof AMR, Harte FM. Effect of dynamic high-pressure treatment on the interfacial and foaming properties of soy protein isolate – hydroxypropylmethylcelluloses systems. Food Hydrocoll. 2011;25(6):1640–5.
31. He X, Liu H, Liu L, Zhao G, Wang Q, Chen Q. Effects of high pressure on the physicochemical and functional properties of peanut protein isolates. Food Hydrocoll. 2014;36:123–9.
32. Zhao Z, Mu T, Zhang M, Richel A. Chemical forces, structure, and gelation properties of sweet potato protein as affected by pH and high hydrostatic pressure. Food Bioprocess Technol. 2018;11(9):1719–32.
33. Yang J, Liu G, Zeng H, Chen L. Effects of high pressure homogenization on faba bean protein aggregation in relation to solubility and interfacial properties. Food Hydrocoll. 2018;83(May):275–86.
34. Katzav H, Chirug L, Okun Z, Davidovich-pinhas M, Shpigelman A. Comparison of thermal and high-pressure gelation of potato protein isolates. Foods. 2020;9(1041):1–19.
35. Zhao S, Li Z, Li N, Zhao Y, Kang Z, Zhu M, et al. Effects of high-pressure processing on the functional properties of pork batters containing Artemisia sphaerocephala krasch gum. Food Eng Mater Sci Nanotechnol. 2021;86(11):4946–57.
36. Pojić M, Misan A, Tiwari B. Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. Trends Food Sci Technol. 2018;75:93–104.
37. Gençdağ E, Görgüç A, Yılmaz FM. Recent advances in the recovery techniques of plant-based proteins from agro-industrial by-products. Food Rev Int. 2020;37(4):1–22.
38. Görgüç A, Bircan C, Yılmaz FM. Sesame bran as an unexploited by-product: effect of enzyme and ultrasound-assisted extraction on the recovery of protein and antioxidant compounds. Food Chem. 2019;283:637–45.
39. Contreras M, Lama-Muñoz A, Gutiérrez-Pérez JM, Espínola F, Moya M, Castro E. Protein extraction from agri-food residues for integration in biorefinery: potential techniques and current status. Bioresour Technol. 2019;280:459–77.
40. Xiong T, Xiong W, Ge M, Xia J, Li B, Chen Y. Effect of high intensity ultrasound on structure and foaming properties of pea protein isolate. Food Res Int. 2018;109:260–7.
41. Žlabur JŠ, Voća S, Dobričević N, Pliestić S, Galić A, Boričević A, et al. Ultrasound-assisted extraction of bioactive compounds from lemon balm and peppermint leaves. Int Agrophys. 2016;30(1):95–104.
42. Vázquez-Espinosa M, González de Peredo AV, Ferreiro-González M, Carrera C, Palma M, Barbero GF, et al. Assessment of ultrasound assisted extraction as an alternative method for the extraction of anthocyanins and total phenolic compounds from maqui berries (Aristotelia chilensis (Mol.) Stuntz). Agronomy. 2019;9(3):1–17.
43. Teng H, Chen L, Huang Q, Wang J, Lin Q, Liu M, et al. Ultrasonic-assisted extraction of raspberry seed oil and evaluation of its physicochemical properties, fatty acid compositions and antioxidant activities. PLoS One. 2016;11(4):1–17.
44. Cavalaro RI, Fabricio LFF, Vieira TMFS. Ultrasound-assisted extraction of antioxidants from Baccharis dracunculifolia and green propolis. Processes. 2020;8(1530):1–11.

45. Silva JPP, Bolanho BC, Stevanato N, Massa TB, Silva C. Ultrasound-assisted extraction of red beet pigments (Beta vulgaris L.): influence of operational parameters and kinetic modeling. J Food Process Preserv. 2020;44(10):1–10.
46. Zhou T, Xu D, Lin S, Li Y, Zheng J, Zhou Y, et al. Ultrasound-assisted extraction and identification of natural antioxidants from the fruit of Melastoma sanguineum Sims. Molecules. 2017;22(2):1–15.
47. Bamba BSB, Shi J, Tranchant CC, Xue SJ, Forney CF, Lim L. Influence of extraction conditions on ultrasound-assisted recovery of bioactive phenolics from blueberry pomace and their antioxidant activity. Molecules. 2018;23(7):1–17.
48. Xu X, Meng J, Mao Q, Shang A, Li B, Zhao C, et al. Effects of tannase and ultrasound treatment on the bioactive compounds and antioxidant activity of green tea extract. Antioxidants. 2019;8(9):362.
49. Alcántara C, Žugcic T, Abdelkebir R, García-Pérez JV, Jambrak AR, Lorenzo JM, et al. Effects of ultrasound-assisted extraction and solvent on the phenolic profile, bacterial growth, and anti-inflammatory/antioxidant activities of mediterranean olive and fig leaves extracts. Molecules. 2020;25:1718.
50. Chen L, Chen J, Ren J, Zhao M. Effects of ultrasound pretreatment on the enzymatic hydrolysis of soy protein isolates and on the emulsifying properties of hydrolysates. J Agric Food Chem. 2011;59:2600–9.
51. Safdar B, Zhihua P, Xinqi L, Jatoi MA, Rashid MT. Influence of different extraction techniques on recovery, purity, antioxidant activities, and microstructure of flaxseed gum. J Food Sci. 2020;85(10):3168–82.
52. Silveira AG, Lopes MMA, Pereira EC, Castro GMC, Germano TA, Oliveira LS, et al. Profile of phenolic compounds and antimicrobial potential of hydroalcoholic extracts from cashew-apple coproducts. Emirates J Food Agric. 2021;33(2):139–48.
53. Alqarni MH, Alam P, Alam A, Ali A, Foudah AI, Alshehri S, et al. A greener hptlc approach for the determination of β-carotene in traditional and ultrasound-based extracts of different fractions of daucus carota (L.), ipomea batatas (l.), and commercial formulation. Agronomy. 2021;11(12):2443.
54. Giordano M, Pinela J, Dias MI, Calhelha RC, Stojković D, Soković M, et al. Ultrasound-assisted extraction of flavonoids from kiwi peel: process optimization and bioactivity assessment. Appl Sci. 2021;11(14):6416.
55. Cui Q, Wang X, Wang G, Li R, Wang X, Chen S, et al. Effects of ultrasonic treatment on the gel properties of microbial transglutaminase crosslinked soy, whey and soy–whey proteins. Food Sci Biotechnol. 2019;28(5):1455–64.
56. Oliveira APH, Omura MH, Barbosa ÉAA, Bressan GC, Vieira ÉNR, Coimbra JSR, et al. Combined adjustment of pH and ultrasound treatments modify techno-functionalities of pea protein concentrates. Colloids Surf A Physicochem Eng Asp. 2020;603(June):125156.
57. Omura MH, Oliveira APH, Soares LS, Coimbra JSR, Barros FAR, Vidigal MCTR, et al. Effects of protein concentration during ultrasonic processing on physicochemical properties and techno-functionality of plant food proteins. Food Hydrocoll. 2021;113:106457.
58. Biswas B, Sit N. Effect of ultrasonication on functional properties of tamarind seed protein isolates. J Food Sci Technol. 2020;57(6):2070–8.
59. Caner C, Yuceer M. Maintaining functional properties of shell eggs by ultrasound treatment. J Sci Food Agric. 2015;95(14):2880–91.
60. Misra NN, Tiwari BK, Raghavarao KSMS, Cullen PJ. Nonthermal plasma inactivation of food-borne pathogens. Food Eng Rev. 2011;3(3–4):159–70.
61. Mao L, Mhaske P, Zing X, Kasapis S, Majzoobi M, Farahnaky A. Cold plasma: microbial inactivation and effects on quality attributes of fresh and minimally processed fruits and ready-to-eat vegetables. Trends Food Sci Technol [Internet]. 2021;116(May):146–75. Available from: https://doi.org/10.1016/j.tifs.2021.07.002.

62. Misra NN, Pankaj SK, Segat A, Ishikawa K. Cold plasma interactions with enzymes in foods and model systems. Trends Food Sci Technol [Internet] 2016;55:39–47. Available from: https://doi.org/10.1016/j.tifs.2016.07.001.
63. Li X, Li M, Ji N, Jin P, Zhang J, Zheng Y, et al Cold plasma treatment induces phenolic accumulation and enhances antioxidant activity in fresh-cut pitaya (Hylocereus undatus) fruit. LWT [Internet]. 2019;115(March):108447. Available from: https://doi.org/10.1016/j.lwt.2019.108447.
64. Wielogorska E, Ahmed Y, Meneely J, Graham WG, Elliott CT, Gilmore BF. A holistic study to understand the detoxification of mycotoxins in maize and impact on its molecular integrity using cold atmospheric plasma treatment. Food Chem [Internet]. 2019;301(February):125281. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0308814619313913.
65. Phan KTK, Phan HT, Boonyawan D, Intipunya P, Brennan CS, Regenstein JM, et al Non-thermal plasma for elimination of pesticide residues in mango. Innov Food Sci Emerg Technol [Internet]. 2018;48(December 2017):164–71. Available from: https://doi.org/10.1016/j.ifset.2018.06.009.
66. Venkataratnam H, Sarangapani C, Cahill O, Ryan CB. Effect of cold plasma treatment on the antigenicity of peanut allergen Ara h 1. Innov Food Sci Emerg Technol [Internet]. 2019;52(February):368–75. Available from: https://doi.org/10.1016/j.ifset.2019.02.001.
67. Grzegorzewski F, Michaela Z, Rohn S, Kroh LWLW, Schlueter O. Modification of polyphenols and cuticular surface lipids of Kale (B. oleracea convar. sabellica) with non-thermal oxygen plasma gaseous species. In: 11th international congress on engineering and food, Athens, Greece. 2011;(Table 1):1–4.
68. Saremnezhad S, Soltani M, Faraji A, Hayaloglu AA. Chemical changes of food constituents during cold plasma processing: a review. Food Res Int [Internet]. 2021;147(June):110552. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0963996921004518.
69. Illera AE, Chaple S, Sanz MT, Ng S, Lu P, Jones J, et al. Effect of cold plasma on polyphenol oxidase inactivation in cloudy apple juice and on the quality parameters of the juice during storage. Food Chem X. 2019;3(August):100049.
70. Dasan BG, Boyaci IH. Effect of cold atmospheric plasma on inactivation of Escherichia coli and physicochemical properties of apple, orange, tomato juices, and sour cherry nectar. Food Bioprocess Technol. 2018;11(2):334–43.
71. Herceg Z, Kovačević DB, Kljusurić JG, Jambrak AR, Zorić Z, Dragović-Uzelac V. Gas phase plasma impact on phenolic compounds in pomegranate juice. Food Chem. 2016;190:665–72.
72. Alves Filho EG, Silva LMA, de Brito ES, Castro DRG, Bezerra JA, Sanches EA, et al. Effect of glow and dielectric barrier discharges plasma on volatile and non-volatile chemical profiling of camu-camu juice. Food Bioprocess Technol. 2021;14(7):1275–86.
73. Campelo PH, Alves Filho EG, Silva LMA, de Brito ES, Rodrigues S, Fernandes FAN. Modulation of aroma and flavor using glow discharge plasma technology. Innov Food Sci Emerg Technol [Internet]. 2020;62(March):102363. Available from: https://doi.org/10.1016/j.ifset.2020.102363.
74. Tappi S, Ramazzina I, Rizzi F, Sacchetti G, Ragni L, Rocculi P. Effect of plasma exposure time on the polyphenolic profile and antioxidant activity of fresh-cut apples. Appl Sci. 2018;8(10):1939.
75. Bao Y, Reddivari L, Huang JY. Enhancement of phenolic compounds extraction from grape pomace by high voltage atmospheric cold plasma. LWT [Internet]. 2020;133(April):109970. Available from: https://doi.org/10.1016/j.lwt.2020.109970.
76. Farias TRB, Alves Filho EG, Silva LMA, De Brito ES, Rodrigues S, Fernandes FAN. NMR evaluation of apple cubes and apple juice composition subjected to two cold plasma technologies. LWT [Internet]. 2021;150(June):112062. Available from: https://doi.org/10.1016/j.lwt.2021.112062.
77. Almeida FDL, Cavalcante RS, Cullen PJ, Frias JM, Bourke P, Fernandes FAN, et al Effects of atmospheric cold plasma and ozone on prebiotic orange juice. Innov Food Sci Emerg Technol [Internet]. 2015;32:127–35. Available from: https://doi.org/10.1016/j.ifset.2015.09.001.

78. Zou JJ, Liu CJ, Eliasson B. Modification of starch by glow discharge plasma. Carbohydr Polym. 2004;55(1):23–6.
79. Zhang B, Chen L, Li X, Li L, Zhang H. Understanding the multi-scale structure and functional properties of starch modulated by glow-plasma: a structure-functionality relationship. Food Hydrocoll [Internet]. 2015;50:228–36. Available from: https://doi.org/10.1016/j.foodhyd.2015.05.002.
80. Thirumdas R, Trimukhe A, Deshmukh RR, Annapure US. Functional and rheological properties of cold plasma treated rice starch. Carbohydr Polym [Internet]. 2017;157:1723–31. Available from: https://doi.org/10.1016/j.carbpol.2016.11.050.
81. Tiwari BK, O'Donnell CP, Patras A, Brunton N, Cullen PJ. Effect of ozone processing on anthocyanins and ascorbic acid degradation of strawberry juice. Food Chem [Internet]. 2009;113(4):1119–26. Available from: https://doi.org/10.1016/j.foodchem.2008.08.085.
82. Marangoni AG. Kinetic model for chlorophyll degradation in green tissue. 2. Pheophorbide degradation to colorless compounds. J Agric Food Chem. 1996;44(12):3735–40.
83. Lafeuille JL, Lefèvre S, Lebuhotel J. Quantitation of chlorophylls and 22 of their colored degradation products in culinary aromatic herbs by HPLC-DAD-MS and correlation with color changes during the dehydration process. J Agric Food Chem. 2014;62(8):1926–35.
84. Matile P, Hörtensteiner S, Thomas H. Chlorophyll degradation. Annu Rev Plant Biol. 1999;50(February):67–95.
85. Nowacka M, Dadan M, Janowicz M, Wiktor A, Witrowa-Rajchert D, Mandal R, et al. Effect of nonthermal treatments on selected natural food pigments and color changes in plant material. Compr Rev Food Sci Food Saf. 2021;20(5):5097–144.
86. Mollakhalili-Meybodi N, Yousefi M, Nematollahi A, Khorshidian N. Effect of atmospheric cold plasma treatment on technological and nutrition functionality of protein in foods. Eur Food Res Technol [Internet]. 2021;247(7):1579–94. Available from: https://doi.org/10.1007/s00217-021-03750-w.
87. Segat A, Misra NN, Cullen PJ, Innocente N. Atmospheric pressure cold plasma (ACP) treatment of whey protein isolate model solution. Innov Food Sci Emerg Technol [Internet] 2015;29:247–54. Available from: https://doi.org/10.1016/j.ifset.2015.03.014.
88. Bahrami N, Bayliss D, Chope G, Penson S, Perehinec T, Fisk ID. Cold plasma: a new technology to modify wheat flour functionality. Food Chem [Internet] 2016;202:247–53. Available from: https://doi.org/10.1016/j.foodchem.2016.01.113.
89. Sifuentes-Nieves I, Mendez-Montealvo G, Flores-Silva PC, Nieto-Pérez M, Neira-Velazquez G, Rodriguez-Fernandez O, et al. Dielectric barrier discharge and radio-frequency plasma effect on structural properties of starches with different amylose content. Innov Food Sci Emerg Technol. 2021;68(October 2020):102630.
90. Misra NN, Martynenko A, Chemat F, Paniwnyk L, Barba FJ, Jambrak AR. Thermodynamics, transport phenomena, and electrochemistry of external field-assisted nonthermal food technologies. Crit Rev Food Sci Nutr. 2018;58(11):1832–63.
91. Nowosad K, Sujka M, Pankiewicz U, Kowalski R. The application of PEF technology in food processing and human nutrition. J Food Sci Technol [Internet]. 2021;58(2):397–411. Available from: https://doi.org/10.1007/s13197-020-04512-4.
92. Dermesonlouoglou E, Zachariou I, Andreou V, Taoukis PS. Effect of pulsed electric fields on mass transfer and quality of osmotically dehydrated kiwifruit. Food Bioprod Process. 2016;100:535–44.
93. Li J, Shi J, Huang X, Zou X, Li Z, Zhang D, et al. Effects of pulsed electric field on freeze-thaw quality of Atlantic salmon. Innov Food Sci Emerg Technol. 2020;65(June):102454.
94. Liu C, Pirozzi A, Ferrari G, Vorobiev E, Grimi N. Impact of pulsed electric fields on vacuum drying kinetics and physicochemical properties of carrot. Food Res Int [Internet]. 2020;137(September):109658. Available from: https://doi.org/10.1016/j.foodres.2020.109658.

95. Wiktor A, Sledz M, Nowacka M, Rybak K, Chudoba T, Lojkowski W, et al. The impact of pulsed electric field treatment on selected bioactive compound content and color of plant tissue. Innov Food Sci Emerg Technol. 2015;30:69–78.
96. Andreou V, Dimopoulos G, Dermesonlouoglou E, Taoukis P. Application of pulsed electric fields to improve product yield and waste valorization in industrial tomato processing. J Food Eng [Internet]. 2020;270(October 2019):109778. Available from: https://doi.org/10.1016/j.jfoodeng.2019.109778.
97. López-Gámez G, Elez-Martínez P, Martín-Belloso O, Soliva-Fortuny R. Enhancing phenolic content in carrots by pulsed electric fields during post-treatment time: Effects on cell viability and quality attributes. Innov Food Sci Emerg Technol [Internet]. 2020;59(August 2019):102252. Available from: https://doi.org/10.1016/j.ifset.2019.102252.
98. Nowacka M, Tappi S, Wiktor A, Rybak K, Miszczykowska A, Czyzewski J, et al. The impact of pulsed electric field on the extraction of bioactive compounds from beetroot. Foods. 2019;8(7):244.
99. El Darra N, Turk MF, Ducasse MA, Grimi N, Maroun RG, Louka N, et al Changes in polyphenol profiles and color composition of freshly fermented model wine due to pulsed electric field, enzymes and thermovinification pretreatments. Food Chem [Internet] 2016;194(2016):944–50. Available from: https://doi.org/10.1016/j.foodchem.2015.08.059.
100. Zhang S, Yang R, Zhao W, Liang Q, Zhang Z. The first ESR observation of radical species generated under pulsed electric fields processing. LWT – Food Sci Technol. 2011;44(4):1233–5.
101. Zhang ZH, Wang LH, Zeng XA, Han Z, Wang MS. Effect of pulsed electric fields (PEFs) on the pigments extracted from spinach (Spinacia oleracea L.). Innov Food Sci Emerg Technol. 2017;43(April):26–34.
102. Giteru SG, Oey I, Ali MA. Feasibility of using pulsed electric fields to modify biomacromolecules: a review. Trends Food Sci Technol. 2018;72(January 2017):91–113.
103. Zhou Y, He Q, Zhou D. Optimization extraction of protein from mussel by high-intensity pulsed electric fields. J Food Process Preserv. 2017;41(3):e12962.
104. Prabhu MS, Levkov K, Livney YD, Israel A, Golberg A. High-voltage pulsed electric field preprocessing enhances extraction of starch, proteins, and ash from marine macroalgae Ulva ohnoi. ACS Sustain Chem Eng. 2019;7(20):17453–63.
105. Wu L, Zhao W, Yang R, Yan W. Pulsed electric field (PEF)-induced aggregation between lysozyme, ovalbumin and ovotransferrin in multi-protein system. Food Chem. 2015;175:115–20.
106. Zhang S, Sun L, Ju H, Bao Z, Zeng X, Lin S. Research advances and application of pulsed electric field on proteins and peptides in food. Food Res Int [Internet]. 2021;139(1):109914. Available from: https://doi.org/10.1016/j.foodres.2020.109914.
107. Zhang L, Wang LJ, Jiang W, Qian JY. Effect of pulsed electric field on functional and structural properties of canola protein by pretreating seeds to elevate oil yield. LWT – Food Sci Technol [Internet]. 2017;84:73–81. Available from: https://doi.org/10.1016/j.lwt.2017.05.048.
108. Zeng F, Gao QY, Han Z, Zeng XA, Yu SJ. Structural properties and digestibility of pulsed electric field treated waxy rice starch. Food Chem [Internet]. 2016;194:1313–9. Available from: https://doi.org/10.1016/j.foodchem.2015.08.104.
109. Hong J, Zeng XA, Han Z, Brennan CS. Effect of pulsed electric fields treatment on the nanostructure of esterified potato starch and their potential glycemic digestibility. Innov Food Sci Emerg Technol. 2018;45(October 2017):438–46.
110. Pataro G, Carullo D, Falcone M, Ferrari G. Recovery of lycopene from industrially derived tomato processing by-products by pulsed electric fields-assisted extraction. Innov Food Sci Emerg Technol. 2020;63(April):102369.
111. Moraes JO, Moraru CI. High-intensity pulsed light processing. In: Proctor A, editor. Alternatives to conventional food processing [internet]. 2nd ed. Royal Society of Chemistry; 2018. p. 355–96. Available from: http://ebook.rsc.org/?DOI=10.1039/9781782626596-00355.
112. Koutchma T. Validation of light-based processes. Valid Food Preserv Process Based Nov Technol. 2022:113–67.

113. Buosi DTM, de Moraes JO, Cheng Y, Cheng RA, Moraru CI, Carciofi BAM. Effective pulsed light treatments for inactivating Salmonella enterica serotypes. Food Control. 2022;135:108776.
114. Verduin J. Photodegradation products and their analysis in food. Food Sci Nutr. 2020;6(3):1–16.
115. Braga TR, Silva EO, Rodrigues S, Fernandes FAN. Drying of mangoes (Mangifera indica L.) applying pulsed UV light as pretreatment. Food Bioprod Process [Internet]. 2019;114:95–102. Available from: https://doi.org/10.1016/j.fbp.2018.11.013.
116. Huyskens-Keil S, Eichholz-Dündar I, Hassenberg K, Herppich WB. Impact of light quality (white, red, blue light and UV-C irradiation) on changes in anthocyanin content and dynamics of PAL and POD activities in apical and basal spear sections of white asparagus after harvest. Postharvest Biol Technol [Internet]. 2020;161(September 2019):111069. Available from: https://doi.org/10.1016/j.postharvbio.2019.111069.
117. FDA. Kinetics of microbial inactivation for alternative food processing technologies -ultraviolet light. Safe Pract Food Process [Internet]. 2014;(2):1–37. Available from: http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm103137.htm.
118. Sng BJR, Mun B, Mohanty B, Kim M, Phua ZW, Yang H, et al. Combination of red and blue light induces anthocyanin and other secondary metabolite biosynthesis pathways in an age-dependent manner in Batavia lettuce. Plant Sci [Internet]. 2021;310(January):110977. Available from: https://doi.org/10.1016/j.plantsci.2021.110977.
119. Tosati JV, de Oliveira EF, Oliveira JV, Nitin N, Monteiro AR. Light-activated antimicrobial activity of turmeric residue edible coatings against cross-contamination of Listeria innocua on sausages. Food Control. 2018;84:177–85.
120. Chen J, Zhang M, Devahastin S. UV-C irradiation-triggered nutritional change of 4D printed ergosterol-incorporated purple sweet potato pastes: conversion of ergosterol into vitamin D2. LWT [Internet]. 2021;150(April):111944. Available from: https://doi.org/10.1016/j.lwt.2021.111944.
121. Avalos-Llano KR, Martín-Belloso O, Soliva-Fortuny R. Effect of pulsed light treatments on quality and antioxidant properties of fresh-cut strawberries. Food Chem [Internet]. 2018;264(May):393–400. Available from: https://doi.org/10.1016/j.foodchem.2018.05.028.
122. Sattar A, de Man JM, Alexander JC. Effect of wavelength on light induced quality deterioration of edible oils and fats. Can Inst Food Sci Technol J [Internet]. 1976;9(3):108–13. Available from: https://doi.org/10.1016/S0315-5463(76)73641-1.
123. Monteiro MLG, Rosário DKA, de Carvalho APA, Conte-Junior CA. Application of UV-C light to improve safety and overall quality of fish: a systematic review and meta-analysis. Trends Food Sci Technol. 2021;116(January):279–89.
124. Silva-Espinoza BA, Palomares-Navarro JJ, Tapia-Rodriguez MR, Cruz-Valenzuela MR, González-Aguilar GA, Silva-Campa E, et al. Combination of ultraviolet light-C and clove essential oil to inactivate Salmonella Typhimurium biofilms on stainless steel. J Food Saf. 2020;40(3):e12788.
125. Ricciardi FE, Plazzotta S, Conte A, Manzocco L. Effect of pulsed light on microbial inactivation, sensory properties and protein structure of fresh ricotta cheese. LWT [Internet]. 2021;139(July 2020):110556. Available from: https://doi.org/10.1016/j.lwt.2020.110556.
126. Elmnasser N, Dalgalarrondo M, Orange N, Bakhrouf A, Haertlé T, Federighi M, et al. Effect of pulsed-light treatment on milk proteins and lipids. J Agric Food Chem. 2008;56(6):1984–91.
127. Siddique MAB, Maresca P, Pataro G, Ferrari G. Effect of pulsed light treatment on structural and functional properties of whey protein isolate. Food Res Int [Internet]. 2016;87:189–96. Available from: https://doi.org/10.1016/j.foodres.2016.07.017.
128. Manzocco L, Panozzo A, Nicoli MC. Effect of pulsed light on selected properties of egg white. Innov Food Sci Emerg Technol [Internet]. 2013;18:183–9. Available from: https://doi.org/10.1016/j.ifset.2013.02.008.

129. Xiaokang W, Brunton NP, Lyng JG, Harrison SM, Carpes ST, Papoutsis K. Volatile and non-volatile compounds of shiitake mushrooms treated with pulsed light after twenty-four hour storage at different conditions. Food Biosci [Internet]. 2020;36(April):100619. Available from: https://doi.org/10.1016/j.fbio.2020.100619.
130. Kurdziel M, Łabanowska M, Pietrzyk S, Pająk P, Królikowska K, Szwengiel A. The effect of UV-B irradiation on structural and functional properties of corn and potato starches and their components. Carbohydr Polym. 2022;289(March):119439.
131. Vykydalová A, Cibulková Z, Čížová K, Vizárová K, Šimon P. Degradation of beeswax by NOx pollution and UV light studied by DSC and FTIR measurements. Thermochim Acta [Internet]. 2020;689(x):178606. Available from: https://doi.org/10.1016/j.tca.2020.178606.

Chapter 11
Toxicological Aspects of Natural Food Additives

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11.1 Introduction

Natural food additives (NFAs) are perceived as innocuous substances and are believed to be healthy to confer added value as biological bioactive compounds. These NFAs are extracted from vegetables, fruits, plants, fungi, algae, and bacteria. Adequate extraction and isolation techniques must be used to preserve the compound and avoid changes in chemical structure, contaminants, and solvents residues. NFAs are usually proteins, lipids, carbohydrates, vitamins, and metabolites to be eliminated from the body through metabolic conversion. Although people prefer to consume foods containing natural additives (NAs) over synthetic ones [1] generally, these compounds are used at higher levels than synthetic food additives (SFAs) to achieve the same technological or organoleptic function; thus, toxicological studies are needed to ensure their safety. Except for natural flavours, there is no definition of natural preservatives, antioxidants, colours, or sweeteners, which shows the growing concern regarding the regulation of NAs since they are widely used,

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especially in ultra-processed food products in Western countries. Conversely, natural flavourings are legislated in the European Union (EU) and the United States of America (USA); however, this legislation must not be extrapolated to other classes of additives.

The safety of food additives is evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) through acute, short-term, sub-chronic, and chronic toxicity studies, as well as carcinogenicity, reproductive, and developmental toxicity studies [2–5]. These toxicological studies are carried out on animals (rodents and non-rodent species), and according to each food additive's nature and potential toxicity, a maximum amount of the food additive contained in the product could be established. However, sometimes those maximum amounts are referred to as "good manufacturing practices" (GMP), which means the product can contain as much as needed to reach the desired effect. The acute toxicity test is the first toxicological study required, the animals are administered once by oral, intramuscular, intraperitoneal, or dermal administration route, and the effects are observed at 24 or 48 h to obtain the median lethal dose (LD_{50}); this value indicates the dose of the food additive that causes 50% of the death of test animals and serves to estimate the potential danger to humans, identify target organs and doses levels to be used for other toxicological studies [4, 6]. Furthermore, food additives can be classified into different toxicity categories based on the LD_{50}, as shown in Table 11.1.

In short-term toxicity studies, oral administration is the most appropriate route, and usually, a food additive is administered repeatedly for 14 or 28 days. These studies are helpful to establish the dose for sub-chronic and chronic toxicity studies [2, 8]. Through the long-term toxicity studies mentioned above, values such as No Observed Adverse Effect Levels (NOAEL) and Lowest Observed Adverse Effect Level (LOAEL) are determined [9]. These values are used to extrapolate to human exposure and calculate the acceptable daily intake (ADI), which is the amount of a food additive that can be consumed daily over a lifetime without appreciable risk to the health of the human population, including sensitive age groups such as children. The ADI is expressed as mg/kg body weight (bw) per day and is calculated by dividing NOAEL or LOAEL by safety factors that consider intraspecies and interspecies variation. The safety factor of 100 is commonly used; it is calculated by multiplying

Table 11.1 Toxicity range classification based on the LD_{50}

LD_{50} (mg/kg body weight)	Category
<5	Extremely toxic
5–50	Highly toxic
50–500	Moderately toxic
500–5000	Slightly toxic
5000–15,000	Practically non-toxic
>15,000	Relative harmless

Adapted from Loomis and Hayes [7]
LD_{50} Median lethal dose

10 by 10; the first safety factor extrapolates from animal to human and the second considers the human-to-human variability response. However, if the toxic effect in animals is severe; another factor 10 is added [2, 3, 9, 10]. It is important to note that if the LOAEL is used to calculate the ADI, an additional safety factor of 2–3 is added to account for the observed adverse effect not considered by the NOAEL [2]. Currently, the WHO guideline suggests subdividing the traditionally 10 factors by 10, the result of multiplying 4 by 2.5, which consider the toxicokinetic and toxicodynamic of the food additive, respectively, these safety factors reflect the difference between humans and species of test and reduce the uncertainty in estimating the ADI [2, 9]. Once established the ADI value, JECFA has to verify if the maximum levels of food additives proposed for a specific technological function are within the ADI values, and then they are made public for consultation [11]. It is unlikely that a person exceeds the ADI because the levels of additives used in foods are minimal, and the person would have to consume a large amount of food. If it were to occur, occasional overconsumption of the food additives would not pose a health risk unless it is over prolonged periods [12]. Moreover, there are food additives, mainly naturally occurring, that have been shown to have low toxicity, and the levels used in foods do not pose a health hazard, so these food additives have an ADI not specified or are considered as generally regarded as safe (GRAS), these NFAs should be used under GMP conditions [11]. The GRAS status is evaluated by the FDA considering published scientific studies of identity and composition, detection methods, use levels, estimated exposure in foods, proposed tolerances, complete toxicity studies reports, and environmental information [4].

The toxicity of NFAs will depend on the levels used in foods, the time of exposure, the species (human or animals), and, above all, the interaction with other food additives or food components. There are few studies focused on the toxicity of NFAs, and most have a non-specified ADI, indicating that their consumption does not have harmful effects on human health. This chapter summarises the toxic effect of some NFAs from the different groups, such as antioxidants, colourants, antimicrobials, and sweeteners (Table 11.2).

11.2 Natural Antioxidants

Antioxidant additives play an essential role in the food industry since they have become one of the most widespread methods for preserving food due to their low cost and ease of use [47]. Antioxidants are mainly used in foods to preserve attributes such as colour, texture, aroma, flavour, and overall product quality by inhibiting the oxidation of lipids, proteins, and pigments [48]. According to their mechanism of action, antioxidants can be classified into (1) primary antioxidants known as radical scavengers or chain-breaking antioxidants; (2) chelators, which bind to metals and prevent them from initiating radical formation; (3) fire extinguishers, which deactivate high-energy oxidising species; (4) oxygen scavengers, which remove oxygen from systems, preventing their destabilization; and (5)

Table 11.2 Natural food additives and toxicological parameters

Additive	Food use levels	Application in food products	Toxicological parameters	Main toxic effects	Reference
Annatto (E160) (colourant)	GMP	Butter, margarine, salad dressing, ice cream, frozen desserts, confectionery, egg products, fish, meat, fruit and bakery, cereals, beverages, soup mixes and snacks	Approved as GRAS ADI_{bixin} = 0–12 mg/kg_{bw}/day $ADI_{norbixin}$ = 0–0.6 mg/kg_{bw}/day	Nd	[13, 14]
Anthocyanins (E163) (colourants)	GMP	Beverages, confections, preserves, fruit products, ice cream, yoghurt, gelatine desserts, candies, and bakery toppings	ADI = 0–2.5 mg/kg_{bw}/day for grape skin extract LD_{50} = 2000 mg/kg_{bw} in mice and rats, oral route	Nd	[15–18]
Carnosic acid and carnosol (antioxidant)	22.5–130 ppm for meat patties 1000 ppm per mg of rosemary extract/kg of meat	Oils, animal fats, sauces, processed potatoes, pastry, meat, fish, and chicken burgers	ADI = 0.3 mg/kg_{bw}/day NOAEL = 64 mg/kg_{bw}/day LD_{50} = 7100 mg/kg_{bw} in mice, oral route	Reversible liver enlargement and hepatocellular hypertrophy Cytotoxicity	[19, 20]
Carminic acid (E120) (colorant)	GMP	Jams, gelatines, baked goods, dairy products, non-carbonated drinks, meat products, seafood, confectionery, alcoholic beverages, vinegar, and yoghurt.	Approved as GRAS ADI = 0–5 mg/kg_{bw}/day	Nd	[21–23]
Carotenoids (E160a) (colorant)	GMP	Sauces, marinades, spice blends, coatings, beverages, custards, yoghurts, processed nuts, precooked pasta and noodles.	ADI not specified	Nd	[24–26]
Chlorophylls (E140) (colorants)		Dairy products, confectionery, desserts, beverages, fruit preparation, bakery products, soups, sauces, snack food, and seasonings.	ADI not specified	Nd	[27, 28]

Curcumin (E100) (colorant)	GMP	Desserts, seasoning, fish paste and crustacean paste, precooked crustaceans, smoked fish, ice creams, processed cheese, salami, meal substitutes and soups.	Approved as GRAS ADI = 3 mg/kg$_{bw}$/day	Nd	[29, 30]
(−)-epigallocatechin-3-gallate (EGCG) (antioxidant)	0–1000 ppm (soybean oil)	Meat, chicken, sausages, fish, cheese, shrimp, oysters, edible oils, bread, biscuits, cakes	ADI not specified	Hepatoxicity	[31, 32]
Erythritol (E968) (sweetener)	1.5–3.5% in non-alcoholic beverage	Cheese products, milk powder, ice cream, breakfast cereals, processed meat products	ADI not specified LD_{50} = 13 g/kg$_{bw}$ in rats, oral route	Nd	[33, 34]
Lysozyme (CAS 9066-59-6) (antimicrobial)	<40 ppm in cheese	Cheese	ADI not specified	Nd	[35]
Nisin (E234) (antimicrobial)	3–12.5 ppm	Unripe cheese, heat-treated meat products	ADI = 1 mg/kg$_{bw}$/day NOAEL = 225 mg/kg$_{bw}$/day	Hepatoxicity	[36–39]
Natamycin (CAS 7681-93-8)(antimicrobial)	6–40 ppm	Cheese and in non-heat-treated dried meats	GRAS status ADI = 0–0.3 mg/kg$_{bw}$/day	Nd	[40]
Steviol glucosides (E960) (sweetener)	10–29 ppm	Sweeten food stuffs and beverages	ADI = 4 mg/kg$_{bw}$/day LD_{50} = 15 g/kg$_{bw}$ in rats and mice, oral route	Mutagenic	[41, 42]
Tocopherols (antioxidant)	0.03%, in animal fats, and 0.02% in mixtures with BHA, BHT and PG	Films, coatings, bacon, meat, dairy products, oils	ADI not specified	Increased risk of prostate cancer	[43, 44]
Thaumatin (E957) (sweetener)	100 ppm	Cola drinks, snacks, food and chocolate industries	ADI = 50 mg/kg$_{bw}$/day LD_{50} > 20 g/kg$_{bw}$ in rats and mice, oral route	Nd	[45, 46]

ADI adequate daily intake, *BHA* butylhydroxyanisole, *BHT* butylhydroxytoluene, *EGCG* (−)-epigallocatechin-3-gallate, *LD_{50}* median lethal dose, *GMP* good manufacturing practices, *GRAS* generally recognised as safe, *Nd* not determined, *NOAEL* non-observed adverse effects levels *PG* propyl gallate, *ppm* parts per million

antioxidant regenerators, which regenerate other antioxidants when they radicalise [49]. Consumer trends have caused the industry to look to natural antioxidants for food preservation, and while SFAs have been mainstream, their natural counterparts have gained interest [47]. Most of these natural compounds have been marked as harmless for the same reason; many have an ADI not specified; therefore, they can be added to food products under GMP conditions [47]. However, it is necessary to find the source of natural antioxidants that is acceptable, safe, with high potential, and biologically active in low concentrations [50].

Polyphenols are one of the types of natural food antioxidants used, among which we can find rosemary extracts (*Rosmarinus officinalis L.*) and green tea extracts (*Camellia sinensis*) [47, 50]. Rosemary extracts have been identified as a food additive with E392. The most important antioxidant effects of rosemary extracts are attributed mainly to the phenolic diterpenes carnosic acid and carnosol (carnosol being the primary oxidised metabolite of carnosic acid) [51]. They are used in oils, animal fats, sauces, processed potatoes, soups, processed nuts, baked goods, fish, meat pies (between 22.5 and 130 ppm for meat pies), and chicken patties (1000 ppm per mg of rosemary extract/kg of meat), among others [19, 52]. Previously, rosemary extracts were considered innocuous; however, the 80-s meeting of the JECFA in 2017 proposed a temporary ADI of 0.3 mg/kg_{bw}/day (expressed as total carnosic acid and carnosol) for rosemary extracts [53]. Among the side effects that have been noted from rosemary extracts are liver alterations. A study in male and female rats treated with acetone (800 mg/kg_{bw}) and supercritical carbon dioxide (300, 600, or 2400 mg/kg_{bw}) extracts of rosemary for 90 days reported liver enlargement and hepatocellular hypertrophy. However, these effects have been declared fully reversible, adaptive, and without toxicological concerns. In this study, the NOAEL was considered to be 64 mg/kg_{bw}/day [54]. Cytotoxic effects have also been observed in the invertebrate *Daphnia manga*; however, those effects have been only reported in very high doses of an ethanolic dry extract of rosemary leaves (at least 500 μg/mL) [20]. Rosemary extract is sometimes added with other antioxidants such as butylhydroxyanisole (BHA; ADI: 0.5 mg/kg_{bw}/day), butylhydroxytoluene (BHT; 0–0.3 mg/kg_{bw}), propyl gallate (PG; 0–1.4 mg/kg_{bw}), and tert-butylhydroxyquinone (TBHQ; 0.7 mg/kg_{bw}) to cause a synergistic effect and enhance the antioxidant effect in foods [55–58]. However, some synthetic antioxidants can cause neurotoxic effects, testicular dysfunction, and hepatoxicity [59–61].

On the other hand, catechins from green tea extract have been used as additives in meat, poultry, sausages, cheeses, fish, shrimp, oysters, and plant food products such as edible oils, bread, cookies, cakes, and noodles [62]. In general, catechins from green tea consumption is considered safe; however, hepatotoxicity cases have been reported in humans, mice, rats, and dogs [31, 63]. The hepatotoxicity of green tea has been related to the presence of (−)-epigallocatechin-3-gallate (EGCG), one of the most abundant catechins in this tea, especially when the intake is equal to or greater than 800 mg/day. For example, consumption of 843 mg/day of EGCG for 12 months induces moderate to severe abnormalities in liver function in about 6.7% of postmenopausal women, which was reflected by increased alanine aminotransferase [64, 65]. There is no evidence of hepatotoxicity in clinical studies using

concentrations below 800 mg of EGCG/day for 12 months [66, 67]. Given the hepatotoxic effects associated with EGCG, an oral LD_{50} value in a range of 190–1900 mg EGCG/kg_{bw} was established in rats [31, 68]. This dose was similar to the LD_{50} for green tea extracts (with 93% EGCG) in Wistar rats, which is reported to be between 200 and 2000 mg/kg_{bw} (186.8 and 1868 mg EGCG/kg_{bw}). This means that a dose of 2000 mg of green tea preparation/kg_{bw} is lethal to rats, while a 200 mg/kg_{bw} dose does not induce toxicity [69]. In addition, a clinical study showed that excessive consumption of green tea (concentration not mentioned in the article) could cause iron deficiency anaemia due to the formation of complexes between this metal and the catechins of green tea [70].

It should be noted that oil-soluble green tea extract (OS-GTE), a green tea preparation used as a food additive, did not present toxic effects and was defined as a GRAS product [71]. OS-GTE is designed to be added to various conventional foods in the US at usage levels ranging from 0.05% to 0.28% (500–2800 ppm) [71]. Furthermore, a study conducted in Sprague-Dawley rats with a 90-day treatment of OS-GTE found the NOAEL to be 0.50 g/kg_{bw}/day, while the 30-day treatment found the NOAEL to be 2.33 g/kg_{bw}/day [72]. Therefore, the European Food Safety Authority (EFSA) has recommended that studies be conducted to determine the dose-response of hepatotoxicity and other toxic effects that green tea catechins might have and to examine interspecies and intraspecies variability. In addition, since green tea extracts are thought to be contaminated with pyrrolizidine alkaloids, maximum limits should be established due to they may contribute to hepatotoxicity. The labels of green tea products must include the content of catechins and the proportion of EGCG [31].

Another type of natural food antioxidant used is that of tocopherols (vitamin E). This group has been identified with the numbers E306–E309. EFSA has only allowed α-, β- and γ-tocopherol to be used as a food additive, leaving out δ-tocopherol [47]. The main target of tocopherols is foods with high lipid content since they are the most powerful lipophilic antioxidants, which is why they have been used in bacon, meat, dairy products, oils, and coatings [47, 49]. Tocopherols have long been reported to have no harmful health effects. In addition, vitamin E is essential for life since it protects the human body's cells against oxidation and helps assimilate vitamin K [73]. An ADI for tocopherols has not yet been established; however, a Tolerable Upper Intake Level (UL) for vitamin E of 300 mg/day for adults has been proposed [43]. As common antioxidants, tocopherols have GRAS status and are considered safe substances added to food. In the USA, common tocopherols are limited to 0.03%, or 300 ppm in animal fats, and 0.02% in blends with BHA, BHT, and PG [44]. Although relatively large amounts of vitamin E generally do not cause harm, isolated cases of toxicity have been reported. For example, in a 3-year double-blind clinical trial, vitamin E treatment (400 IU/d of all rac-α-tocopheryl acetate) was reported to significantly increase the risk of prostate cancer among healthy men [74]. However, the UK Group of Experts on Vitamins and Minerals established a NOAEL for humans (age not defined) of 540 mg of α-tocopherol equivalents for vitamin E supplements, which is equivalent to 9.0 mg/kg_{bw}/day in a 60 kg adult [75].

Another antioxidant widely used as a food additive is L-ascorbic acid (vitamin C) and its salts (sodium ascorbate and calcium ascorbate), identified with E300-E304. Ascorbic acid is one of the most widespread antioxidants in food [44, 47]. Ascorbic acid and ascorbate salts are used in practically every country globally and have GRAS status with no limits on use [44]. They are used in lipid oils, dry fermented sausages, dairy products, cured or cooked meat products. All ascorbate additives have ADI not determined [47]. As indicated in the literature, vitamin C is protected at supplementation levels of up to 600 mg/day, with an upper intake limit of up to 2000 mg/day [44].

Furthermore, ascorbic acid is usually added in combination with synthetic antioxidants such as BHT and BHA to further increase their antioxidant capacity, given the excellent properties of ascorbic acid to regenerate other antioxidants. It is also associated with tocopherols since it helps to regenerate them [47]. To date, no short-term and long-term toxic effects of ascorbic acid are known [76].

11.3 Natural Colorants

Colour is one of the most relevant organoleptic attributes that confers attractive, appealing, appetising, and informative features influencing consumers' acceptance and food selection. For this reason, the industry adds colourants to enhance existing colours, prevent colour loss during the manufacture or over the shelf life, or attribute new colours to the final product [77].

The FDA defines a colour additive as any dye, pigment, or other substance that can impart colour to a food, drug, cosmetic, or the human body; however, this definition does not differentiate between natural or synthetic colour additive. Most natural colourants are extracts derived from plants, which leads to problems such as varying colour intensity, instability upon exposure to light or heat, traces from the extraction process, reactivity with other food components, and addition of secondary sources flavours and odours. Although there are many dyes, we will review some of the most used.

Anthocyanins identified as E163 are obtained from vegetables and edible fruits by maceration or extraction with sulphite water, carbon dioxide or methanol/ ethanol. These anthocyanins are responsible for pigments such as red, purple, violet, and they are used for colour beverages, confectionery, desserts, ice cream, fruit preparations, bakers jam, yoghurt, gelatine desserts, candy, and bakery fillings and toppings, among others [78]. There are no reports about the adverse health effect of anthocyanins, the LD_{50} in mice and rats is 2000 mg/$kg_{bw,}$ and the ADI is 0–2.5 mg/kg_{bw}/day. However, it describes that anthocyanin can produce sedative effects in animals at 5000 mg/kg_{bw}, but as usually consumed in the diet, they are safe for human consumption [15].

Annatto is a permitted natural food colourant extracted from the *Bixa Orellana L.* tree and labelled as E160b. Annatto mixture is mainly constituted by carotenoids bixin and norbixin, which display dark red solutions, emulsions, or dark red

powders, also offering a wide spectrum of colours from yellow to orange. Annatto is highly used in cakes (from 250 to 1000 mg/kg of dough), butter, margarine, salad dressing, ice cream and other frozen desserts, confectionery, egg products, fish and fish products, meat products, cereals, cakes, beverages, soup mixes, fruit products, snacks, bakery products, spices and seasonings, and sausage casings [79]. In 2013, the scientific opinion from EFSA Panel concluded that further research should be carried out due to a lack of toxicological data, and ADI for bixin was established to 0–12 mg/kg_{bw}/day and ADI for norbixin and its disodium and dipotassium salts of 0–0.6 mg/kg_{bw}/day expressed as norbixin [16].

Another food colourant highly used is paprika, mainly extracted from *Capsicum annuum L.*, and the mixture of two carotenoids, capsanthin and capsorubin, displaying an orange to red colour. Paprika is labelled as E160c and approved in the EU and is used to colour meat products, confectionery, vegetable oils, snacks, surimi, marinades, seasonings, salad dressings, soups, sauces, bakery products, and processed cheese, among others. The EU has established an ADI of 24 mg/kg_{bw}/day for paprika extract [21].

There are many other carotenoids used in food, namely β-carotene, lutein, violaxanthin, neoxanthin, β-cryptoxanthin, fucoxanthin, lycopene, and astaxanthin, as well as other carotenes identified as E160a(i), E160a(ii), E160a(iii), and E160a(iv) in EU.

Carotenes are mainly obtained from carrots (*Daucus carota*), oil of palm fruit (*Elaeis guinensis*), sweet potato (*Ipomoea batatas*), saffron (*Crocus sativus*). However, they could also be extracted from algae and even insects, representing a broad spectrum of colours in the food industry. The main applications of carotenoids in food are related to sauces, marinades, spice blends, coatings, beverages, milk, beverages including cider, margarine, cheeses, cake fillings, custards, yoghurts, processed nuts, precooked pasta, and noodles [24].

Curcumin identified as E100 is a pigment purified from turmeric, which is extracted from the dried rhizomes of the plant *Curcuma longa L.* This dye confers an orange colour to food products such as mustard, yoghurt, baked goods, dairy industry, fine bakery products, desserts, seasoning, fish paste and crustacean paste, precooked crustaceans, smoked fish, ice creams, processed cheese, salami, meal substitutes, soups, dried potato granules and flakes, jams, and salad dressings. Curcumin (E100) has very low toxicity, has not shown genotoxicity [80], and the ADI is 3 mg/kg_{bw}/day [29]. There are currently six food categories for which maximum permitted levels for curcumin have been adopted in the General Standard of Food Additives [81].

Carminic acid labelled as E120 is a dominant pigment in the insect *Dactylopius coccus Costa*, and a brilliant red colour could be obtained after mixing with aluminium. However, due to is mainly extracted from this insect is relatively expensive when compared to other red pigments since about 100,000 are needed to obtain 1 kg of product, although its quality, efficiency and stability are elevated. It is used in jams, gelatines, baked goods, dairy products, non-carbonated drinks, meat products, seafood, confectionery, alcoholic beverages, vinegar, and yoghurts [82, 83]. Currently, there are more than 70 food categories for which maximum permitted

levels for carmines have been implemented in the General Standard of Food Additives (GSFA). Since 2000, the ADI for cochineal, carminic acid, and carmine (E120) has been limited to 5 mg/kg_{bw}/day [21]. Currently, the acute, short-term, sub-chronic carcinogenicity, reproduction, and developmental toxicity studies conducted in rats or mice did not show any toxicological potential [84].

The chloroplasts of higher plants contain chlorophyll a and b, which are insoluble in water, sensitive to light, pH, oxygen, and heat. Thus, the instability of chlorophyll, with the code E140(i), limits its use as a food additive, despite the absence of toxicity for the amounts usually consumed [27]. However, chlorophyllins E140(ii) and chlorophylls derivates are water-soluble, and although the polarity is high, the stability is still poor, and the industry prefers lipid-soluble cupric chlorophyll E141(i) and water-soluble chlorophyllin complexes E141(ii). These derivatives result from replacing Mg^{2+} by Cu^{2+} atoms in the chlorophyll molecule, stabilising the molecule and improving colour stability for longer. E140 and E141 are used in dairy products, desserts, beverages, dairy products, ice cream, fruit preparation, sauces, bakery products, snack food, drinks, soups, and sugar confections [82]. Since chlorophylls occur naturally in plants at relatively high concentrations, its consumption as a food additive is not of safety concern since the consumption is higher through the diet than as an additive [27].

11.4 Natural Antimicrobials

Various additives are used in the food industry to prevent microbial contamination and foodborne illnesses, such as food poisoning caused by *Staphylococcus aureus* or *Bacillus cereus* toxins [85]. Some natural antimicrobial additives, also named biopreservatives, are from bacterial, fungi, plants, and animals origin [86].

Currently, the only natural additives with antimicrobial activity found in JECFA are nisin, lysozyme and natamycin [49, 87, 88]. Nisin labelled as E234 is an antimicrobial peptide, also known as a bacteriocin, produced mainly by *Lactococcus lactis*, although other bacteria such as *Staphylococcus capitias* and *Blautia obeum* could also produce it [89, 90]. This natural antimicrobial is used in dairy products and meat to avoid the growth of several relevant contaminants in the food industry, such as *Listeria monocytogenes*, *Staphylococcus aureus, Clostridium sp. and Bacillus sp.* [36]. It is used in different concentrations from 3 to 12.5 ppm of product, and it should be considered that one international unit (UI) corresponds to 0.25 μg of nisin [91]. This natural antimicrobial has GRAS status with NOAEL of 224.7 mg/kg_{bw} [92]; since its oral administration for 13 weeks at different concentrations in a rat model has no effects on histology of pituitary gland, thyroids, heart, liver, kidney, reproductive organs, saliva glands and with minimal hyperplasia in forestomach [38, 39]. Thus, JECFA established an ADI of 0–2 mg/kg_{bw}/day [93]. However, an increasing approach focuses on the gastrointestinal microbiota, and previously nisin was reported not to affect the composition of the gut microbiota

[94]. However, a deeper analysis in rat faeces shows that nisin consumption in different starch matrices reduces *Bifidobacterium*, but increases the *Escherichia/Shigella* genera [95]. In addition, nisin consumption reduces the *Staphylococcus*, *Rodentibacter*, *Rothia*, and *Lactobacillus* genera in the oral cavity of rats, increasing *Acinetobacter*, *Aerococcus*, and *Pseudomonas* genera; moreover, the levels of immunoglobulin A in saliva are reduced [96]. Although there are currently no reports of nisin toxicity, it is essential to point out that its effects on microbiota could be relevant in gastrointestinal infection and other pathologies with dysbiosis such as obesity [97] and chronic kidney disease [98].

Lysozyme (CAS 9066-59-6), also known as muramidase, is a polypeptide produced by most vertebrates and found in several tissues and serum [99]. In the food industry, the primary source of lysozyme is the chicken egg, which is often used mainly in cheese and wine [96]. In acute toxicity studies in rats and mice, it was found that oral administration of lysozyme was tolerated up to 4000 mg/kg_{bw} in mice and rats. At the same time, tolerance by intravenous administration was up to 1000 mg/kg_{bw} in mice and up to 2000 mg/kg_{bw} in rats and rabbits [35]. Although lysozyme is considered safe [56], some harmful effects have been shown, for instance, is a potential allergen [100] and, in the case of chemically modified lysozyme by the Maillard reaction, it produces advanced glycation end products (AGEs), which has been reported to cause diet-induced inflammatory responses [101].

Another natural antimicrobial used in food products is natamycin (CAS 7681-93-8)**,** also known as pimaricin; it is an antifungal agent produced by *Streptomyces natalensis*, *S. chattanogenesis, S. gilvosporeous and, S. lydicus*. This molecule has a broad range of action against yeast and mold, such as *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii*, *Aspergillus sp*., *Penicillium sp*., *Mucor sp*., *Fusarium* sp., among others [102]. This antifungal agent is used in dairy products, sausages, juices, and wines, and the acceptable levels are from 6 to 40 ppm [102]. Although natamycin currently has GRAS status, the LD_{50} has been reported to be in more than 450 mg/kg_{bw} in different species [102]. Different studies assure the consumption of pimaricin, reflecting that it has no negative effects on reproduction nor carcinogenic or mutagenic potential. However, studies carried out in rabbits and in men with doses higher than 500 mg/kg_{bw}/day and 300–400 mg/kg_{bw}/day of pimaricin, respectively, report effects such as diarrhoea, nausea and vomiting. In the case of the study conducted with rabbits, it was reported that the animals that died presented haemorrhagic gastric mucosa. While in the case of the studies conducted with males, it is reported that gastrointestinal symptoms begin to appear from 5 mg/kg_{bw}/day. Therefore, JECFA established an ADI of 0–0.3 mg/kg_{bw}/day [40, 102].

The natural antimicrobial epsilon-poly-L-lysine possesses a broad range of activity against bacteria, mould, and yeast [103]. This molecule can be synthesised by chemosynthesis or by a great diversity of bacteria, with *Streptomyces albulus* being the most commonly used for industrial production [104]. The use of epsilon-poly-l-lysine in food range from 10 to 500 ppm in boiled rice, noodles, soup stock, and cooked vegetables, while 1000–5000 ppm is used in fish [105]. Nowadays, epsilon-poly-L-lysine is considered non-toxic and possesses the GRAS status [106].

However, a study in pigs reveals that its consumption alters microbiota in the ileum and reduces the digestibility [107], having an anti-obesogenic effect [108], although this could have a potential risk in persons with gastrointestinal alterations, such inflammatory bowel disease [109].

11.5 Natural Sweeteners

Since excessive sugar consumption has been linked to the development of chronic metabolic diseases prevalent in Western countries, low-caloric or no-caloric sweeteners have been an alternative in the human diet due to their low caloric content. These sweeteners can be natural or artificial in origin. The application of natural sweeteners has grown, as they are considered safer than artificial sweeteners. However, an additive of natural origin is not strictly safe. Consequently, natural sweeteners have been subjected to toxicological studies to ensure their consumption. However, studies investigating the safety of sweeteners in the short- and long-term periods are controversial about the effects on human health [45, 110] as well as noting that most of the studies carried out on the effect of the consumption of natural sweeteners that reflect a toxicological impact on health were carried out in the 1960 and 1990s. Therefore, in this section, we will focus on the toxicological effects of natural sweeteners.

Steviol glycosides (E960) are alternative natural non-caloric sweeteners obtained mainly from the leaves of the shrub *Stevia rebaudiana* Bertoni [111, 112]. Nine steviol glycosides have been identified in the leaves of S. rebaudiana: stevioside, rebaudiosides A-F, dulcoside A, and steviolbioside [113, 114]. Steviol glycone (steviol) is the major metabolite of stevioside by the hepatic and intestinal microbiota of humans and rats [41, 115]. Comparative toxicological studies show that stevioside does not display toxicity, while steviol could be mutagenic [41, 42, 116, 117]. In addition, previous studies have shown that steviol has very low acute oral toxicity with an $LD_{50} > 15$ g/kg_{bw} for rats and mice, and in hamsters were 5.20 and 6.10 g/kg_{bw} for males and females, respectively. However, nephrotoxic has also been observed in the proximal tubules of the renal cortex of hamsters [41, 118, 119]. Matsui et al. [117] reported that steviol with metabolic activation could be mutagenic, inducing chromosomal breakage in the Chinese hamster lung fibroblast cell line at 1.0–1.5 mg/mL [117]. Likewise, Nunes et al. [116] reported that administration of stevioside at 4 mg/mL for 45 days in drinking water resulted in lesions in chromosomal DNA in blood cells, spleen, liver, and brain in Wistar rats [116]. On the other hand, toxicity evaluations have been performed from stevia leaf compounds obtained by aqueous extraction; but a study by Qiannan Zhang et al. [120] performed with stevia leaf compounds extracted by ethanolic extraction showed no genotoxicity effects but low cytotoxicity by oral exposure. Since the evidence is not conclusive, JECFA determined in 2019 that there are no safety concerns about steviol glycosides, indicating that the temporary ADI for steviol is 0–4 mg/kg_{bw}/day [121].

Sugar alcohols or polyols are a group of low-calorie natural sweeteners; within this group, tagatose (E963), xylitol (E967), and erythritol (E968) are found. In general, subchronic and chronic toxicity studies conducted in rats, mice, dogs, and humans, and *in vitro* studies conducted to assess mutagenicity, clastogenicity, embryotoxicity have reflected the safety of sugar alcohol consumption [122–126]. Excessive consumption's main adverse health effects are gastrointestinal problems such as vomiting, gas, or laxative effects [45, 127]. The LD_{50} determined by oral route for erythritol in male and female rats is 13,100 and 13,500, respectively, and <5000 for male dogs [45, 125, 128]. In addition, the erythritol maximum tolerated dose in 184 children aged from 4 to 6 years was determined after the administration by non-carbonated beverages, showing that ingestion of 15 g (0 0.73 g/kg_{bw} for both sexes, 0.72 g/kg_{bw} for boys, and 0.76 g/kg_{bw} for girls) was well tolerated with no severe gastrointestinal alterations [129]. Although doses of xylitol as low as 0.15 g/kg_{bw} in dogs can cause life-threatening hypoglycaemia and acute liver failure, no such adverse effects have been reported in humans, indicating that xylitol is not well tolerated in dogs [125]. Consequently, JECFA has assigned an ADI of not specified due to its very low toxicity for this type of sweeteners [130].

Additionally, sweet proteins such as thaumatin (E957) and monellin are alternative sources of low caloric natural sweeteners; however, their toxicological effects on health are relatively low [131, 132]. Since no adverse effects were observed in subchronic studies in rats and dogs at the highest dose tested up to 5200 and 1476 mg/kg_{bw}/day, respectively, the thaumatin is currently considered safe and authorised by regulatory agencies for consumption and sale in the EU [46, 133]. Thus, the LD50 established is >20,000 mg/kg_{bw} in rats and mice [134]. Similar results have been found in human studies, observing the lack of toxicity from oral exposure to thaumatin in 15 subjects at 280 mg thaumatin/day to thaumatin for 13 weeks [46]. Consequently, the ADI for humans has been evaluated as not specified [46, 133]. Based on a study by A. Hagiwara et al., thaumatin was administered orally to Sprague-Dawley rats of both sexes for 13 weeks; they revealed that thaumatin exerted no adverse effect. Thus, the NOAEL was considered a dietary level of at least 3.0% (2502 mg/kg_{bw}/day for males, 2889 mg/kg_{bw}/day for females) [135].

11.6 Conclusions

In this chapter, we summarised and analysed the current toxicological information about some NFAs from the different groups, such as antioxidants, colourants, antimicrobials, and sweeteners. In general, NFAs tend to be safe; indeed, several of the NFAs do not have an established ADI and most of them are approved as GRAS by the FDA. Additionally, the NOAEL reported is usually a very high dose indicating their safety. However, to ensure the food safety, the toxicity of NFAs must be evaluated periodically as well as those products using labels such as "all natural additives" or "no synthetic additives", particularly because the toxic effects in humans could emerge after several years of consumption and years after the toxicological

evaluation was approved. Moreover, although the toxic effects mainly depend on the dose, humans do not only consume a single additive, but a mixture of food additives and other compounds, and there are genetic and environmental factors that may or may not enhance the toxic effects of the additives.

In addition, *in vitro*, *in vivo,* and epidemiological information should be considered to state a conclusion about the toxicity induced by NFAs and demonstrate their safety since the data obtained from those different models draw different conclusions. Moreover, the continuous development of new techniques and technological and scientific advances make it possible to evaluate with greater precision the changes at the genetic, immunological and microbiome levels, that were previously not detected. It is also worthy to mention, that toxicological parameters such as NOAEL, LOAEL, LD_{50}, and ADI are reference values that estimate potential toxicity and the adequate doses for human consumption. However, these are not absolute toxicological values since factors such as the specie model used, gender differences, purity of the compounds, doses and time for evaluation could impact in the measurable toxicological parameters.

Conflicts of Interest The authors declare no conflict of interest.

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References

1. Carocho M, Barreiro MF, Morales P, Ferreira ICFR. Adding molecules to food, pros and cons: a review on synthetic and natural food additives. Compr Rev Food Sci Food Saf. 2014;13:377–99. https://doi.org/10.1111/1541-4337.12065.
2. Barlow SM. Toxicology of food additives. In: Ballantyne B, Marrs TC, Syversen TLM, editors. General and applied toxicology. Chichester: John Wiley & Sons, Ltd; 2009.
3. Güngörmüş C, Kılıç A. The safety assessment of food additives by reproductive and developmental toxicity studies. In: El-Samragy Y, editor. Food additive. InTech; 2012.
4. Pressman P, Clemens R, Hayes W, Reddy C. Food additive safety. Toxicol Res Appl. 2017;1:239784731772357. https://doi.org/10.1177/2397847317723572.
5. WHO: Safety evaluation of certain food additives: prepared by the eighty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2017. https://apps.who.int/iris/handle/10665/258934.
6. Erhirhie EO, Ihekwereme CP, Ilodigwe EE. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdiscip Toxicol. 2018;11:5–12. https://doi.org/10.2478/intox-2018-0001.
7. Loomis TA, Hayes AW. Loomis's essentials of toxicology. 4th ed. Academic Press; 1996.
8. Guy RC. Redbook (redbook 2000: toxicological principles for the safety of food ingredients). In: Wexler P, editor. Encyclopedia of toxicology. Elsevier; 2014. p. 61–2.
9. Chilakapati J, Mehendale HM. Acceptable daily intake (ADI). In: Wexler P, editor. Encyclopedia of toxicology. Elsevier; 2014. p. 8–9.

10. Gilsenan MB. Additives in dairy foods | safety. In: Fox PF, Fuquay JW, McSweeney PLH, editors. Encyclopedia of dairy sciences. Elsevier; 2011. p. 55–60.
11. Tomaska LD, Brooke-Taylor S. Food additives: food additives – general. In: Motarjemi Y, Moy G, Todd ECD, editors. Encyclopedia of food safety. Elsevier; 2014. p. 449–54.
12. Ilbäck N-G, Busk L. Food additatives. Use, intake and safety. Näringsforskning. 2000;44:141–9. https://doi.org/10.3402/fnr.v44i0.1778.
13. EFSA. The safety of annatto extracts (E 160b) as a food additive. EFSA J. 2016;14:e04544. https://doi.org/10.2903/j.efsa.2016.4544.
14. CFR: Anatto. https://www.ecfr.gov/current/title-21/chapter-I/subchapter-A/part-73/subpart-C/section-73.2030.
15. Burton-Freeman B, Sandhu A, Edirisinghe I. Anthocyanins. In: Gupta R, editor. Nutraceuticals. Elsevier; 2016. p. 489–500.
16. EFSA. Scientific opinion on the re-evaluation of anthocyanins (E 163) as a food additive. EFSA J. 2013;11:3145. https://doi.org/10.2903/j.efsa.2013.3145.
17. JECFA: Toxicological evaluation of certain food additives. http://apps.who.int/iris/bitstream/handle/10665/41546/WHO_TRS_683.pdf;jsessionid=6080748100026DB9EEB2ECB26E12C8071?sequence=1.
18. CFR: Grape color extract. https://www.ecfr.gov/current/title-21/chapter-I/subchapter-A/part-73/subpart-A/section-73.169.
19. Chammem N, Saoudi S, Sifaoui I, Sifi S, de Person M, Abderraba M, Moussa F, Hamdi M. Improvement of vegetable oils quality in frying conditions by adding rosemary extract. Ind Crop Prod. 2015;74:592–9. https://doi.org/10.1016/j.indcrop.2015.05.054.
20. Gîrd CE, Nencu I, Popescu ML, Costea T, Duțu LE, Balaci TD, Olaru OT. Chemical, antioxidant and toxicity evaluation of rosemary leaves and its dry extract. Farmacia. 2017;65:978–83.
21. EFSA. Scientific opinion on the re-evaluation of cochineal, carminic acid, carmines (E 120) as a food additive. EFSA J. 2015;13:4288. https://doi.org/10.2903/j.efsa.2015.4288.
22. JECFA: 55th Report of the JECFA. Evaluation of certain food additives and contaminants. https://www.who.int/ipcs/publications/jecfa/reports/trs940.pdf.
23. CFR: Cochineal extract; carmine. https://www.ecfr.gov/current/title-21/chapter-I/subchapter-A/part-73/subpart-A/section-73.100.
24. IACM: Carotenes. https://iacmcolor.org/color-profile/carotenoids/.
25. JECFA: Evaluation of certain food additives and contaminants: forty-first report of the Joint FAO/WHO Expert Committee on Food Additives (meeting held in Geneva from 9 to 18 February 1993). https://apps.who.int/iris/handle/10665/36981.
26. EFSA. Scientific opinion on the re-evaluation of mixed carotenes (E 160a (i)) and beta-carotene (E 160a (ii)) as a food additive. EFSA J. 2012;10:2593. https://doi.org/10.2903/j.efsa.2012.2593.
27. EFSA. Scientific opinion on the re-evaluation of chlorophylls (E 140(i)) as food additives. EFSA J. 2015;13:4089. https://doi.org/10.2903/j.efsa.2015.4089.
28. IACM. International Association of Color Manufacturers. Chlorophylls. Available on: https://iacmcolor.org/color-profile/chlorophylls-and-chlorophyllins/
29. EFSA. Scientific opinion on the re-evaluation of curcumin (E 100) as a food additive. EFSA J. 2010;8:1679. https://doi.org/10.2903/j.efsa.2010.1679.
30. JECFA: Evaluation of certain food additives and contaminants. https://apps.who.int/iris/handle/10665/42849.
31. EFSA, Younes M, Aggett P, Aguilar F, Crebelli R, Dusemund B, Filipič M, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Lambré C, Leblanc J, Lillegaard IT, Moldeus P, Mortensen A, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Andrade RJ, Fortes C, Mosesso P, Restani P, Arcella D, Pizzo F, Smeraldi C, Wright M. Scientific opinion on the safety of green tea catechins. EFSA J. 2018;16:e05239. https://doi.org/10.2903/j.efsa.2018.5239.

32. Cao Y, Ai N, True AD, Xiong YL. Effects of (−)-epigallocatechin-3-gallate incorporation on the physicochemical and oxidative stability of myofibrillar protein–soybean oil emulsions. Food Chem. 2018;245:439–45. https://doi.org/10.1016/j.foodchem.2017.10.111.
33. EFSA. Scientific opinion on the safety of the proposed extension of use of erythritol (E 968) as a food additive. EFSA J. 2015;13:4033. https://doi.org/10.2903/j.efsa.2015.4033.
34. Gültekin F. Food additives and microbiota. North. Clin. Istanbul. 2019;7:192–200. https://doi.org/10.14744/nci.2019.92499.
35. WHO: Lysozyme (WHO food additives series 828). 1992.
36. Özel B, Şimşek Ö, Akçelik M, Saris PEJ. Innovative approaches to nisin production. Appl Microbiol Biotechnol. 2018;102:6299–307. https://doi.org/10.1007/s00253-018-9098-y.
37. EFSA, Younes M, Aggett P, Aguilar F, Crebelli R, Dusemund B, Filipič M, Frutos MJ, Galtier P, Gundert-Remy U, Kuhnle GG, Lambré C, Leblanc J, Lillegaard IT, Moldeus P, Mortensen A, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Herman L, Tobback P, Pizzo F, Smeraldi C, Tard A, Papaioannou A, Gott D. Safety of nisin (E 234) as a food additive in the light of new toxicological data and the proposed extension of use. EFSA J. 2017;15:5063. https://doi.org/10.2903/j.efsa.2017.5063.
38. Hagiwara A, Imai N, Nakashima H, Toda Y, Kawabe M, Furukawa F, Delves-Broughton J, Yasuhara K, Hayashi S. A 90-day oral toxicity study of nisin A, an anti-microbial peptide derived from Lactococcus lactis subsp. lactis, in F344 rats. Food Chem Toxicol. 2010;48:2421–8. https://doi.org/10.1016/j.fct.2010.06.002.
39. Reddy KVR, Gupta SM, Aranha CC. Effect of antimicrobial peptide, Nisin, on the reproductive functions of rats. ISRN Vet Sci. 2011;2011:1–8. https://doi.org/10.5402/2011/828736.
40. WHO. Evaluation of certain food additives and contaminants: thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organ – Tech Rep Ser. 1990;789:7–37.
41. Brusick DJ. A critical review of the genetic toxicity of steviol and steviol glycosides. Food Chem Toxicol. 2008;46:S83–91. https://doi.org/10.1016/j.fct.2008.05.002.
42. Moore J. 84th JECFA – Chemical and Technical Assessment (CTA), 2017 © FAO 2017 STEVIOL GLYCOSIDES Chemical and Technical Assessment (CTA). Revised by Madduri V. Rao, Ph.D. and reviewed by Daniel Folmer, Ph.D. Revised by Jeff Moore for the 82nd JECFA R. 2017.
43. EFSA. Scientific opinion on the re-evaluation of tocopherol-rich extract (E 306), α-tocopherol (E 307), γ-tocopherol (E 308) and δ-tocopherol (E 309) as food additives. EFSA J. 2015;13:4247. https://doi.org/10.2903/j.efsa.2015.4247.
44. Manchanda S, Chandra A, Bandopadhyay S, Deb PK, Tekade RK. Formulation additives used in pharmaceutical products. In: Tekade R, editor. Dosage form design considerations. Elsevier; 2018. p. 773–831.
45. Ruiz-Ojeda FJ, Plaza-Díaz J, Sáez-Lara MJ, Gil A. Effects of sweeteners on the gut microbiota: a review of experimental studies and clinical trials. Adv Nutr. 2019;10:S31–48. https://doi.org/10.1093/advances/nmy037.
46. EFSA Panel on Food Additives and Flavourings (FAF), Younes M, Aquilina G, Castle L, Engel K, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Gundert-Remy U, Husøy T, Manco M, Mennes W, Passamonti S, Moldeus P, Shah R, Waalkens-Berendsen I, Wölfle D, Wright M, Batke M, Boon P, Bruzell E, Chipman J, Crebelli R, Fitzgerald R, Fortes C, Halldorsson T, LeBlanc J, Lindtner O, Mortensen A, Ntzani E, Wallace H, Civitella C, Horvath Z, Lodi F, Tard A, Vianello G. Re-evaluation of thaumatin (E 957) as food additive. EFSA J. 2021;19:e06884. https://doi.org/10.2903/j.efsa.2021.6884.
47. Carocho M, Morales P, Ferreira ICFR. Antioxidants: reviewing the chemistry, food applications, legislation and role as preservatives. Trends Food Sci Technol. 2018;71:107–20. https://doi.org/10.1016/j.tifs.2017.11.008.
48. Ribeiro JS, Santos MJMC, Silva LKR, Pereira LCL, Santos IA, da Silva Lannes SC, da Silva MV. Natural antioxidants used in meat products: a brief review. Meat Sci. 2019;148:181–8. https://doi.org/10.1016/j.meatsci.2018.10.016.

49. Carocho M, Morales P, Ferreira ICFR. Natural food additives: quo vadis? Trends Food Sci Technol. 2015;45:284–95. https://doi.org/10.1016/j.tifs.2015.06.007.
50. Gramza-Michałowska A, Kmiecik D. Functional aspects of antioxidants in traditional food. In: Kristbergsson K, Ötles S, editors. Functional properties of traditional foods. Boston: Springer US; 2016. p. 3–7.
51. Loussouarn M, Krieger-Liszkay A, Svilar L, Bily A, Birtić S, Havaux M. Carnosic acid and carnosol, two major antioxidants of rosemary, act through different mechanisms. Plant Physiol. 2017;175:1381–94. https://doi.org/10.1104/pp.17.01183.
52. Rajeev PS, Johannah NM, Gopakumar G, Maliakel B, Krishnakumar IM. Optimization of antioxidant efficacy of a deflavored and decolorized rosemary extract: effect of carnosol content on the oxidative stability of paprika colored beef patties. J Food Sci Technol. 2017;54:1665–77. https://doi.org/10.1007/s13197-017-2599-3.
53. JECFA: Rosemary extract. In: Safety Evaluation of Certain Food Additives. Eighty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 7–16, 2016. Rome/Geneva: Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO); 2017. p. 123–79, 491, 493.
54. Phipps KR, Lozon D, Baldwin N. Genotoxicity and subchronic toxicity studies of supercritical carbon dioxide and acetone extracts of rosemary. Regul Toxicol Pharmacol. 2021;119:104826. https://doi.org/10.1016/j.yrtph.2020.104826.
55. Guo Q, Gao S, Sun Y, Gao Y, Wang X, Zhang Z. Antioxidant efficacy of rosemary ethanol extract in palm oil during frying and accelerated storage. Ind Crop Prod. 2016;94:82–8. https://doi.org/10.1016/j.indcrop.2016.08.032.
56. EFSA. Scientific opinion related to a notification from the Oenological Products and Practices International Association (OENOPPIA) on lysozyme from hen's egg to be used in the manufacture of wine as an anti-microbial stabilizer/additive pursuant to Article 6. EFSA J. 2011;9:2386. https://doi.org/10.2903/j.efsa.2011.2386.
57. EFSA. Scientific opinion on the re-evaluation of butylated hydroxytoluene BHT (E 321) as a food additive. EFSA J. 2012;10:2588. https://doi.org/10.2903/j.efsa.2012.2588.
58. EFSA. Statement on the refined exposure assessment of tertiary-butyl hydroquinone (E 319). EFSA J. 2016;14:4363. https://doi.org/10.2903/j.efsa.2016.4363.
59. Ham J, Lim W, You S, Song G. Butylated hydroxyanisole induces testicular dysfunction in mouse testis cells by dysregulating calcium homeostasis and stimulating endoplasmic reticulum stress. Sci Total Environ. 2020;702:134775. https://doi.org/10.1016/j.scitotenv.2019.134775.
60. Ham J, Lim W, Park S, Bae H, You S, Song G. Synthetic phenolic antioxidant propyl gallate induces male infertility through disruption of calcium homeostasis and mitochondrial function. Environ Pollut. 2019;248:845–56. https://doi.org/10.1016/j.envpol.2019.02.087.
61. Park S, Lee J-Y, Lim W, You S, Song G. Butylated Hydroxyanisole exerts neurotoxic effects by promoting cytosolic calcium accumulation and endoplasmic reticulum stress in astrocytes. J Agric Food Chem. 2019;67:9618–29. https://doi.org/10.1021/acs.jafc.9b02899.
62. Nikoo M, Regenstein JM, Ahmadi Gavlighi H. Antioxidant and antimicrobial activities of (−)-epigallocatechin-3-gallate (EGCG) and its potential to preserve the quality and safety of foods. Compr Rev Food Sci Food Saf. 2018;17:732–53. https://doi.org/10.1111/1541-4337.12346.
63. Brown AC. Liver toxicity related to herbs and dietary supplements: online table of case reports. Part 2 of 5 series. Food Chem Toxicol. 2017;107:472–501. https://doi.org/10.1016/j.fct.2016.07.001.
64. Dostal AM, Samavat H, Bedell S, Torkelson C, Wang R, Swenson K, Le C, Wu AH, Ursin G, Yuan J-M, Kurzer MS. The safety of green tea extract supplementation in postmenopausal women at risk for breast cancer: results of the Minnesota Green Tea Trial. Food Chem Toxicol. 2015;83:26–35. https://doi.org/10.1016/j.fct.2015.05.019.
65. Yu Z, Samavat H, Dostal AM, Wang R, Torkelson CJ, Yang CS, Butler LM, Kensler TW, Wu AH, Kurzer MS, Yuan J-M. Effect of green tea supplements on liver enzyme elevation: results

from a randomized intervention study in the United States. Cancer Prev Res. 2017;10:571–9. https://doi.org/10.1158/1940-6207.CAPR-17-0160.
66. Pezeshki A, Safi S, Feizi A, Askari G, Karami F. The effect of green tea extract supplementation on liver enzymes in patients with nonalcoholic fatty liver disease. Int J Prev Med. 2016;7:28. https://doi.org/10.4103/2008-7802.173051.
67. Ullmann U, Haller J, Decourt JD, Girault J, Spitzer V, Weber P. Plasma-kinetic characteristics of purified and isolated green tea catechin epigallocatechin gallate (EGCG) after 10 days repeated dosing in healthy volunteers. Int J Vitam Nutr Res. 2004;74:269–78. https://doi.org/10.1024/0300-9831.74.4.269.
68. Emoto Y, Yoshizawa K, Kinoshita Y, Yuki M, Yuri T, Yoshikawa Y, Sayama K, Tsubura A. Green tea extract-induced acute hepatotoxicity in rats. J Toxicol Pathol. 2014;27:163–74. https://doi.org/10.1293/tox.2014-0007.
69. Isbrucker RA, Bausch J, Edwards JA, Wolz E. Safety studies on epigallocatechin gallate (EGCG) preparations. Part 1: genotoxicity. Food Chem Toxicol. 2006;44:626–35. https://doi.org/10.1016/j.fct.2005.07.005.
70. Fan FS. Iron deficiency anemia due to excessive green tea drinking. Clin Case Rep. 2016;4:1053–6. https://doi.org/10.1002/ccr3.707.
71. Kemin Industries, I. d/b/a K.F.T., FDA: GRAS notice for oil-soluble green tea extract (green tea catechin palmitate). 2018.
72. Liu Z, Liu D, Cheng J, Mei S, Fu Y, Lai W, Wang Y, Xu Y, Vo TD, Lynch BS. Lipid-soluble green tea extract: genotoxicity and subchronic toxicity studies. Regul Toxicol Pharmacol. 2017;86:366–73. https://doi.org/10.1016/j.yrtph.2017.04.004.
73. Lidon F, Silva M. An overview on applications and side effects of antioxidant food Additives. Emir J Food Agric. 2016;28:823. https://doi.org/10.9755/ejfa.2016-07-806.
74. Klein EA, Thompson IM, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, Minasian LM, Ford LG, Parnes HL, Gaziano JM, Karp DD, Lieber MM, Walther PJ, Klotz L, Parsons JK, Chin JL, Darke AK, Lippman SM, Goodman GE, Meyskens FL, Baker LH. Vitamin E and the risk of prostate cancer. JAMA. 2011;306:1549. https://doi.org/10.1001/jama.2011.1437.
75. EVM. Safe upper levels for vitamins and minerals. London, UK: Food Standards Agency; 2003.
76. EFSA. Scientific opinion on the re-evaluation of ascorbic acid (E 300), sodium ascorbate (E 301) and calcium ascorbate (E 302) as food additives. EFSA J. 2015;13:4087. https://doi.org/10.2903/j.efsa.2015.4087.
77. Dey S, Nagababu BH. Applications of food color and bio-preservatives in the food and its effect on the human health. Food Chem Adv. 2022;1:100019. https://doi.org/10.1016/j.focha.2022.100019.
78. Sigurdson GT, Tang P, Giusti MM. Natural colorants: food colorants from natural sources. Annu Rev Food Sci Technol. 2017;8:261–80. https://doi.org/10.1146/annurev-food-030216-025923.
79. IACM: Annatto extracts. https://iacmcolor.org/color-profile/annatto-extract/.
80. Aggarwal ML, Chacko KM, Kuruvilla BT. Systematic and comprehensive investigation of the toxicity of curcuminoid-essential oil complex: a bioavailable turmeric formulation. Mol Med Rep. 2016;13:592–604. https://doi.org/10.3892/mmr.2015.4579.
81. FAO/OMS: Curcumin (100(i)). https://www.fao.org/gsfaonline/additives/details.html?id=107.
82. MacDougall DB. Colour in food. Improving quality. Colour food, vol. 388. Woodhead Publishing, CRC Press; 2002.
83. IACM: Carmines. https://iacmcolor.org/color-profile/carmines/.
84. Silva MM, Reboredo FH, Lidon FC. Food colour additives: a synoptical overview on their chemical properties, applications in food products, and health side effects. Foods. 2022;11:379. https://doi.org/10.3390/foods11030379.

85. Forghani F, Wei S, Oh D. A rapid multiplex real-time PCR high-resolution melt curve assay for the simultaneous detection of Bacillus cereus, Listeria monocytogenes, and Staphylococcus aureus in Food. J Food Prot. 2016;79:810–5. https://doi.org/10.4315/0362-028X.JFP-15-428.
86. Pisoschi AM, Pop A, Georgescu C, Turcuş V, Olah NK, Mathe E. An overview of natural antimicrobials role in food. Eur J Med Chem. 2018;143:922–35. https://doi.org/10.1016/j.ejmech.2017.11.095.
87. Cao Y, Liu H, Qin N, Ren X, Zhu B, Xia X. Impact of food additives on the composition and function of gut microbiota: a review. Trends Food Sci Technol. 2020;99:295–310. https://doi.org/10.1016/j.tifs.2020.03.006.
88. Inchem: Monografías del JECFA. https://inchem.org/pages/jecfa.html.
89. Gharsallaoui A, Oulahal N, Joly C, Degraeve P. Nisin as a food preservative: part 1: physicochemical properties, antimicrobial activity, and main uses. Crit Rev Food Sci Nutr. 2016;56:1262–74. https://doi.org/10.1080/10408398.2013.763765.
90. Małaczewska J, Kaczorek-Łukowska E. Nisin—a lantibiotic with immunomodulatory properties: a review. Peptides. 2021;137:170479. https://doi.org/10.1016/j.peptides.2020.170479.
91. Younes M, Aggett P, Aguilar F, Crebelli R, Dusemund B, Filipič M, Frutos MJ, Galtier P, Gundert-Remy U, Kuhnle GG, Lambré C, Leblanc J, Lillegaard IT, Moldeus P, Mortensen A, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Woutersen RA, Wright M, Herman L, Tobback P, Pizzo F, Smeraldi C, Tard A, Papaioannou A, Gott D. Safety of nisin (E 234) as a food additive in the light of new toxicological data and the proposed extension of use. EFSA J. 2017;15:e05063. https://doi.org/10.2903/j.efsa.2017.5063.
92. WHO. Evaluation of certain food additives and contaminants. World Health Organ Tech Rep Ser. 2013;983:1–75., back cover.
93. WHO. Evaluation of certain food additives and contaminants. World Health Organ – Tech Rep Ser. 1983;696:25–9.
94. Bernbom N, Licht TR, Brogren C-H, Jelle B, Johansen AH, Badiola I, Vogensen FK, Nørrung B. Effects of Lactococcus lactis on composition of intestinal microbiota: role of nisin. Appl Environ Microbiol. 2006;72:239–44. https://doi.org/10.1128/AEM.72.1.239-244.2006.
95. Gough R, Cabrera Rubio R, O'Connor PM, Crispie F, Brodkorb A, Miao S, Hill C, Ross RP, Cotter PD, Nilaweera KN, Rea MC. Oral delivery of nisin in resistant starch based matrices alters the gut microbiota in mice. Front Microbiol. 2018;9:1186. https://doi.org/10.3389/fmicb.2018.01186.
96. Wu T, Jiang Q, Wu D, Hu Y, Chen S, Ding T, Ye X, Liu D, Chen J. What is new in lysozyme research and its application in food industry? A review. Food Chem. 2019;274:698–709. https://doi.org/10.1016/j.foodchem.2018.09.017.
97. Vamanu E, Rai SN. The link between obesity, microbiota dysbiosis, and neurodegenerative pathogenesis. Diseases. 2021;9:45. https://doi.org/10.3390/diseases9030045.
98. Feng Z, Wang T, Dong S, Jiang H, Zhang J, Raza HK, Lei G. Association between gut dysbiosis and chronic kidney disease: a narrative review of the literature. J Int Med Res. 2021;49:030006052110532. https://doi.org/10.1177/03000605211053276.
99. Jiang L, Li Y, Wang L, Guo J, Liu W, Meng G, Zhang L, Li M, Cong L, Sun M. Recent insights into the prognostic and therapeutic applications of lysozymes. Front Pharmacol. 2021;12:767642. https://doi.org/10.3389/fphar.2021.767642.
100. Mainente F, Simonato B, Pasini G, Franchin C, Arrigoni G, Rizzi C. Hen egg white lysozyme is a hidden allergen in Italian commercial ciders. Food Addit Contam Part A. 2016;34:1–7. https://doi.org/10.1080/19440049.2016.1265673.
101. Teodorowicz M, Hendriks WH, Wichers HJ, Savelkoul HFJ. Immunomodulation by processed animal feed: the role of Maillard reaction products and advanced glycation end-products (AGEs). Front Immunol. 2018;9:2088. https://doi.org/10.3389/fimmu.2018.02088.
102. Meena M, Prajapati P, Ravichandran C, Sehrawat R. Natamycin: a natural preservative for food applications—a review. Food Sci Biotechnol. 2021;30:1481–96. https://doi.org/10.1007/s10068-021-00981-1.

103. Yoshida T, Nagasawa T. ε-Poly-l-lysine: microbial production, biodegradation and application potential. Appl Microbiol Biotechnol. 2003;62:21–6. https://doi.org/10.1007/s00253-003-1312-9.
104. Wang L, Zhang C, Zhang J, Rao Z, Xu X, Mao Z, Chen X. Epsilon-poly-L-lysine: recent advances in biomanufacturing and applications. Front Bioeng Biotechnol. 2021;9:748976. https://doi.org/10.3389/fbioe.2021.748976.
105. Chheda AH, Vernekar M. A natural preservative ε-poly-L-lysine: fermentative production and applications in food industry. Int Food Res J. 2015;22:23–30.
106. Hiraki J, Ichikawa T, Ninomiya S, Seki H, Uohama K, Seki H, Kimura S, Yanagimoto Y, Barnett JW. Use of ADME studies to confirm the safety of ε-polylysine as a preservative in food. Regul Toxicol Pharmacol. 2003;37:328–40. https://doi.org/10.1016/S0273-2300(03)00029-1.
107. Zhang X, Hou Z, Xu B, Xie C, Wang Z, Yu X, Wu D, Yan X, Dai Q. Dietary supplementation of ε-Polylysine beneficially affects ileal microbiota structure and function in ningxiang pigs. Front Microbiol. 2020;11:544097. https://doi.org/10.3389/fmicb.2020.544097.
108. Kido Y, Hiramoto S, Murao M, Horio Y, Miyazaki T, Kodama T, Nakabou Y. ε-Polylysine inhibits pancreatic lipase activity and suppresses postprandial hypertriacylglyceridemia in rats. J Nutr. 2003;133:1887–91. https://doi.org/10.1093/jn/133.6.1887.
109. Kilby K, Mathias H, Boisvenue L, Heisler C, Jones JL. Micronutrient absorption and related outcomes in people with inflammatory bowel disease: a review. Nutrients. 2019;11:1388. https://doi.org/10.3390/nu11061388.
110. Moriconi E, Feraco A, Marzolla V, Infante M, Lombardo M, Fabbri A, Caprio M. Neuroendocrine and metabolic effects of low-calorie and non-calorie sweeteners. Front Endocrinol (Lausanne). 2020;11:444. https://doi.org/10.3389/fendo.2020.00444.
111. Vasconcelos MA, Orsolin PC, Silva-Oliveira RG, Nepomuceno JC, Spanó MA. Assessment of the carcinogenic potential of high intense-sweeteners through the test for detection of epithelial tumor clones (warts) in Drosophila melanogaster. Food Chem Toxicol. 2017;101:1–7. https://doi.org/10.1016/j.fct.2016.12.028.
112. Grembecka M. Natural sweeteners in a human diet. Rocz Panstw Zakl Hig. 2015;66:195–202.
113. Ceunen S, Geuns JMC. Steviol glycosides: chemical diversity, metabolism, and function. J Nat Prod. 2013;76:1201–28. https://doi.org/10.1021/np400203b.
114. Steurs G, Moons N, Van Meervelt L, Meesschaert B, De Borggraeve WM. Characterization of microbial degradation products of steviol glycosides. Molecules. 2021;26:6916. https://doi.org/10.3390/molecules26226916.
115. Purkayastha S, Kwok D. Metabolic fate in adult and pediatric population of steviol glycosides produced from stevia leaf extract by different production technologies. Regul Toxicol Pharmacol. 2020;116:104727. https://doi.org/10.1016/j.yrtph.2020.104727.
116. Nunes APM, Ferreira-Machado SC, Nunes RM, Dantas FJS, De Mattos JCP, Caldeira-de-Araújo A. Analysis of genotoxic potentiality of stevioside by comet assay. Food Chem Toxicol. 2007;45:662–6. https://doi.org/10.1016/j.fct.2006.10.015.
117. Matsui M, Matsui K, Kawasaki Y, Oda Y, Noguchi T, Kitagawa Y, Sawada M, Hayashi M, Nohmi T, Yoshihira K, Ishidate M, Sofuni T. Evaluation of the genotoxicity of stevioside and steviol using six in vitro and one in vivo mutagenicity assays. Mutagenesis. 1996;11:573–9. https://doi.org/10.1093/mutage/11.6.573.
118. Toskulkac C, Chaturat L, Temcharoen P, Glinsukon T. Acute toxicity of stevioside, a natural sweetener, and its metabolite, steviol, in several animal species. Drug Chem Toxicol. 1997;20:31–44. https://doi.org/10.3109/01480549709011077.
119. Panel E, Additives F, Sources N. Scientific opinion on the safety of the proposed amendment of the specifications for steviol glycosides (E 960) as a food additive. EFSA J. 2015;13:4316. https://doi.org/10.2903/j.efsa.2015.4316.
120. Zhang Q, Yang H, Li Y, Liu H, Jia X. Toxicological evaluation of ethanolic extract from Stevia rebaudiana Bertoni leaves: genotoxicity and subchronic oral toxicity. Regul Toxicol Pharmacol. 2017;86:253–9. https://doi.org/10.1016/j.yrtph.2017.03.021.

121. WHO: Sweetening agent: Stevioside (WHO food additives series 42). 2022.
122. Lina BAR, Bos-Kuijpers MHM, Til HP, Bär A. Chronic toxicity and carcinogenicity study of erythritol in rats. Regul Toxicol Pharmacol. 1996;24:S264–79. https://doi.org/10.1006/rtph.1996.0108.
123. Munro I, Bernt W, Borzelleca J, Flamm G, Lynch B, Kennepohl E, Bär E, Modderman J. Erythritol: an interpretive summary of biochemical, metabolic, toxicological and clinical data. Food Chem Toxicol. 1998;36:1139–74. https://doi.org/10.1016/S0278-6915(98)00091-X.
124. Carocho M, Morales P, Ferreira ICFR. Sweeteners as food additives in the XXI century: a review of what is known, and what is to come. Food Chem Toxicol. 2017;107:302–17. https://doi.org/10.1016/j.fct.2017.06.046.
125. Eapen AK, de Cock P, Crincoli CM, Means C, Wismer T, Pappas C. Acute and sub-chronic oral toxicity studies of erythritol in beagle dogs. Food Chem Toxicol. 2017;105:448–55. https://doi.org/10.1016/j.fct.2017.04.049.
126. Wölnerhanssen BK, Meyer-Gerspach AC, Beglinger C, Islam MS. Metabolic effects of the natural sweeteners xylitol and erythritol: a comprehensive review. Crit Rev Food Sci Nutr. 2020;60:1986–98. https://doi.org/10.1080/10408398.2019.1623757.
127. Tetzloff W, Dauchy F, Medimagh S, Carr D, Bär A. Tolerance to subchronic, high-dose ingestion of erythritol in human volunteers. Regul Toxicol Pharmacol. 1996;24:S286–95. https://doi.org/10.1006/rtph.1996.0110.
128. Mäkinen KK. Gastrointestinal disturbances associated with the consumption of sugar alcohols with special consideration of xylitol: scientific review and instructions for dentists and other health-care professionals. Int J Dent. 2016;2016:1–16. https://doi.org/10.1155/2016/5967907.
129. Jacqz-Aigrain E, Kassai B, Cornu C, Cazaubiel J-M, Housez B, Cazaubiel M, Prével J-M, Bell M, Boileau A, de Cock P. Gastrointestinal tolerance of erythritol-containing beverage in young children: a double-blind, randomised controlled trial. Eur J Clin Nutr. 2015;69:746–51. https://doi.org/10.1038/ejcn.2015.4.
130. Additives F. Evaluation of certain food additives. Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organ – Tech Rep Ser. 1980;648:39–42. https://doi.org/10.1016/0015-6264(81)90537-x.
131. Kant R. Sweet proteins – potential replacement for artificial low calorie sweeteners. Nutr J. 2005;4:5. https://doi.org/10.1186/1475-2891-4-5.
132. Cancelliere R, Leone S, Gatto C, Mazzoli A, Ercole C, Iossa S, Liverini G, Picone D, Crescenzo R. Metabolic effects of the sweet protein MNEI as a sweetener in drinking water. A pilot study of a high fat dietary regimen in a rodent model. Nutrients. 2019;11:2643. https://doi.org/10.3390/nu11112643.
133. Higginbotham JD, Snodin DJ, Eaton KK, Daniel JW. Safety evaluation of thaumatin (talin protein). Food Chem Toxicol. 1983;21:815–23. https://doi.org/10.1016/0278-6915(83)90218-1.
134. WHO: Thaumatin (WHO food additives series 20). 2022.
135. Hagiwara A, Yoshino H, Sano M, Kawabe M, Tamano S, Sakaue K, Nakamura M, Tada M, Imaida K, Shirai T. Thirteen-week feeding study of thaumatin (a natural proteinaceous sweetener), sterilized by electron beam irradiation, in Sprague–Dawley rats. Food Chem Toxicol. 2005;43:1297–302. https://doi.org/10.1016/j.fct.2005.04.001.

Chapter 12
Consumer Attitudes Toward Natural Food Additives

Sibel Bolek

12.1 Introduction

Modern food processing includes the use of several food improvement agents [1]. Among them, consumers are most familiar with food additives (FAs) that are ingredients added to foods aiming to improve their freshness, color, flavor, texture, and nutritional value [2]. Some of the FAs may be extracted from naturally occurring materials; others are manufactured by the chemical industry. All FAs must have a proven useful purpose and pass a rigorous scientific safety evaluation before they can be approved for use. Hence, these FAs have crucial importance in the food supply [3].

Every day, consumers are faced with many food choices that contain FAs. Most of the FAs used by the food industry are also found naturally in foods that people eat routinely. However, most consumers are not aware of these benefits of FAs, so they are skeptical, although FAs provide important benefits to consumers. Most consumers see FAs as a major threat [4]. Numerous surveys revealed that consumers express concerns about their daily diet, and they are worried about being exposed to synthetic food additives (SFAs) [5, 6]. Natural, homemade foods with local, kitchen cupboard ingredients, are the standard narrative targeted by many consumers, unfortunately they have a very short shelf-life. Therefore, communications aimed at enabling consumers to make informed decisions regarding FAs should be planned meticulously and include key issues relating to consumers' perspectives on risk [7]. Sustaining high standards of food safety, selection, and convenience food supply would not be possible without FAs [8]. Most additives are only allowed in certain foods, and they are subject to certain quantitative limits. Each food additive is

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Table 12.1 Food additives and their functions

Food additive	Function
Antimicrobial, antibrowning, and antioxidant	Preservatives
Vitamins and minerals	Nutritional Supplements
Sweeteners, flavor enhancers, and other flavors	Flavoring agents
Carotenoids, green dyes, blue dyes etc.	Colorings
Stabilizers and emulsifiers	Texturing agents
Enzymes, catalyzers, solvents, and propellants	Miscellaneous (antioxidants, antimicrobials, and stabilizers)

Adapted from Güngörmüş & Kılıç [11]

expressed with a code number determined by the European Union (EU) [9, 10]. FAs and their functions are given in Table 12.1.

The perception of health risk in FAs prevents consumers from understanding the role of these ingredients in food safety and forming a proper behavior towards FAs. Moreover, it brings with it a series of negative effects such as reducing the trust in both processed food and the government [12]. Before approval a new food additive, extensive risk assessments are generally carried out based on animal studies, and FAs currently in use are periodically reassessed [13]. Unfortunately, even these strict measures controlling for additive use have not raised the confidence of average consumers in additives [14]. Consumers with lower education levels rely more on government agencies to regulate FAs. Therefore, reducing the general public's fears of food, strengthening government regulations, or communicating through government officials can have a positive effect on people with low education [15]. Taking into consideration individual gains, worries, and past experiences of consumers can be helpful in dealing with risk perception [16]. Tarnavölgyi [17] investigated consumers' attitudes towards FAs using focus group, being analyzed people with high education levels (doctorate degree), people without high education level, and food industry experts by qualitative market research methods. The results of their study stated that consumers have a variety of concerns pertaining to the health effects of FAs. According to the results, it was suggested that informational campaigns might decrease these concerns. In another study, Esfahani et al. [18] investigated attitudes, knowledge, and practices of university personnel. Their study demonstrated that the personnel had poor attitudes, knowledge, and practices about FAs. These results were explained by insufficient education and training in terms of FAs and interpretation of food labels. In the same line, Ismail et al. [19] investigated the level of awareness of the use of additives in processed foods among consumers. They found a general lack of knowledge about the functions of the broadly used additives in processed foods. In another research, Sachithananthan [20] investigated consumer awareness of FAs in packaged food. They found that majority of the consumers had awareness about the presence of FAs in the packed foods that they buy, but they lacked any specific knowledge about the effects of these additives. Hence, communications aimed at enabling consumers to make informed decisions

regarding FAs should be carefully designed and include key issues from a risk-related viewpoint and from consumers' perspectives [21]. The attention is given by consumers to food safety issues and healthy eating can specify the potential future of any food ingredient [22–25].

Results of previous surveys suggested that gender is a critical factor pertaining to risk perception. Women were more sensitive to potential food risks [26, 27]. Also, men and women may differ in food value (e.g., importance of healthiness, taste, calorie), which may affect the predictors of acceptance of food colors or sweeteners. Different categories of FAs including preservatives, food colorants, or sweeteners may be perceived differently because they serve different aims and consumers benefit from their use to varying degrees [28].

As consumers increasingly become more conscious of natural ingredients, the increasing importance of naturalness among consumers has meant significant ramifications for the food industry [29]. Devcich et al. [30] stated that consumers are becoming more and more knowledgeable about FAs and prefer natural food additives (NFAs) in place of SFAs. Naturalness seems like an important feature for consumers. As unnatural foods are associated with health risks, natural foods are regarded as safe or even healthy [31]. This chapter aimed to analyze consumer attitudes toward NFAs and the factors affecting these attitudes.

12.2 Regulation of Food Additives

Before approving FAs, intensive risk assessment is required [32]. Historically, the safety of FAs has been evaluated based on the risk assessment paradigm defined by the World Health Organization (WHO). The Community legislation on FAs relies on the principle that only additives that are on the list of authorized FAs should be used. It is an important principle of the legislation that consumers are not misled about the use of additives in foods. It is recommended that all FAs be used in limited quantities in foodstuffs [33]. The safety evaluation requires the assessment of data on carcinogenicity, toxicokinetic, genotoxicity, and subchronic/chronic toxicity [34]. Each target jurisdiction/country's regulatory authority uses its own regulatory framework, and while definitions, regulations, and approval processes differ between all target countries, there are many similarities in general. Prior to use in food, all FAs must be authorized, and each is given the same "E-number" to simplify and harmonize labeling items [35]. In any case, the main objective of each authority is to establish and maintain/enforce a regulatory framework to ensure that the food consumed and sold in their country is safe [36]. Categories of FAs and the percentage of their use are given in Table 12.2.

Table 12.2 Categories of food additives and percentage of their use

Additive	Cereals and Cereal Products (%)	Candy and chocolate (%)	Beverages (%)	Dairy and Meat Products (%)	Total (%)
Emulsifiers and Stabilizers	41.09	89.24	44.44	73.56	70.73
Natural coloring	37.21	8.37	63.89	11.85	20.94
Artificial coloring	10.08	28.29	19.44	14.00	20.94
Chemical Leavening Agents	29.46	14.34	Ni	Ni	15.81
Preservatives	10.08	Ni	75.00	22.00	11.11
Flavorings	62.79	94.42	88.89	37.85	78.85
Anti-caking agents	18.60	Ni	Ni	2.00	5.34
Flavor enhancers	16.28	Ni	Ni	5.56	5.13
Humectants	6.98	4.38	Ni	Ni	4.27
Sweeteners	Ni	3.59	19.44	4.00	3.85
Gelling agents	Ni	6.37	Ni	Ni	3.42
Antioxidants	0.78	Ni	19.44	7.56	2.56
Thickeners	0.78	1.20	5.56	1.85	1.50
Glazing agents	Ni	Ni	Ni	2.00	0.21

Ni Not informed
Adapted from Lorenzoni et al. [37]

12.3 Natural Food Additives

Health risk perceptions of FAs were generally associated with their artificiality. Naturalness is a quality that enhances the positive perception of foods and makes these products more attractive than the corresponding unnatural products [38–40]. NFAs are compounds, groups of compounds, or essential oils. NFAs have come a long way from their beginnings. Algae, fungi, and seaweeds are great sources of NFAs. These herbs and their extracts, which have started to attract attention due to changes in consumption habits, are also evaluated in terms of health benefits and combined effects [41].

12.4 Consumers' Perceptions of Natural Food Additives

For several decades, NFAs have received more attention from both the public and food manufacturers. The use of NFAs is steadily increasing because consumers associate SFAs with diseases [42]. Since consumers are hesitant to accept SFAs, naturalness is of crucial importance for consumers. NFAs are considered safe while SFAs are related to health risks [31, 43, 44]. Aiming to increase sales, the food industry has used this perception to its advantage by replacing SFAs with NFAs [45]. The personal importance of the naturalness of foods is crucial in the decision

to accept FAs and perception of risk and benefit. On the other hand, generally, consumers choose foods with no additives [41]. When a type of food or technology is unknown, consumers tend to exaggerate the risk and minimize the risk in familiar foods or in-home preparation [46]. Although NFAs generally do not provide more benefits than SFAs, in most cases they are considered to be healthier, perform various functions in food and provide added value (bioactivity, nutraceutical). Consumers evaluate the naturalness of foods based on their personal perceptions rather than the actual content of a food item [47]. Rozin et al. [48] stated that participants preferred natural foods and drugs to their artificial counterparts irrespective of healthfulness.

E-numbers are considered clues for the lack of naturalness. FAs with E-number are perceived unhealthier compared with only presenting the chemical [49]. It has been known for a long time that the risk perception level of women is higher than that of men. For example, women are more anxious about environmental and technological risks including nuclear waste and gene technology [50]. Similarly, women in Europe were more concerned than men about the health effects of chemicals [51]. When it comes to foods, women were found more anxious than men for risks such as additives, bacteria, oils, and pesticides [52, 53]. Roininen et al. [54] stated that women are more interested than men in the health and natural aspects of food. Similarly, Dickson-Spillmann et al. [55] and Schifferstein and Ophuis [56] observed greater numbers of women in consumer groups more interested in eating a natural diet or in customer groups of organic food stores.

12.4.1 Consumers' Perceptions of Natural Antioxidants

Antioxidants prevent the autoxidation process in various ways, depending on their antioxidant mechanism and structure [57]. Due to their food safety aspects affecting food choices, food antioxidants are one of the fundamental topics of food science [58]. They have a critical role in maintaining the overall quality of products [59]. Synthetic antioxidants, such as some FAs, whose effects on human health are controversial and associated with potential public health risks. Therefore, there is a tendency to use NFAs instead of SFAs. However, synthetic antioxidants are widely used due to their stability, high availability, and low cost [60]. Some examples of synthetic antioxidants are BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), and TBHQ (tert-Butylhydroquinone). Nowadays, these synthetic antioxidants are going through a difficult time because they have a negative perception in consumers [61]. Acceptable daily intake (ADI) quantities of SFAs are given in Table 12.3.

Since natural antioxidants are relatively unstable, various synthetic antioxidants are used to stabilize food items [62]. The advantages and disadvantages of natural and synthetic antioxidants are summarized in Table 12.4. The natural antioxidants are mainly derived from medicinal plants and foods such as vegetables, fruits, cereals, mushrooms, beverages, spices, flowers, and traditional medicinal herbs

Table 12.3 Acceptable daily intake (ADI) quantities of synthetic food additives

Name	ADI	E number	Legislation
Butylated hydroxyanisole (BHA)	0.5 mg/kg bw	E320	Code of Federal Regulations 21 Sec.175.110 EU Regulation No. 1129/2011
Butylated hydroxytoluene (BHT)	0.05 mg/kg bw	E321	Code of Federal Regulations 21 Sec.175.115 EU Regulation No. 1129/2011
tert-butylhydroquinone (TBHQ)	0.7 mg/kg bw	E319	Code of Federal Regulations 21 Sec.172185 EU Regulation No. 1129/2011
Ethoxyquin (EQ)	0.005 mg/kg bw	E224	Code of Federal Regulations 21 Sec.172.140 EU Regulation No. 1129/2011
Propyl galate (PG)	1.4 mg/kg bw	E310	Code of Federal Regulations 21 Sec.184.1660 EU Regulation No. 1129/2011

bw body weight; *EU* European Union
Adapted from Carocho et al. [41]

Table 12.4 Advantages and disadvantages of natural and synthetic antioxidants

Natural antioxidants	Synthetic antioxidants
Expensive	Cheap
High antioxidant activity	Moderate to high antioxidant activity
Perceived as safe substances	Increasing safety concern
Usage of some products restricted	Widely applied
Fully metabolized	Some of them are stored in adipose tissue
Increasing usage and expanding applications	Some are banned
Wide range of solubilities	Low water solubility
Increasing interest	Decreasing interest

Adapted from Sarkar & Ghosh [74]

[63–65]. Aiming to apply natural colorants with antioxidant properties in foods, Caleja et al. [66] compared the effects of natural and synthetic antioxidants on biscuits and concluded that the combination of natural and synthetic additives did not change the color or nutritional value of biscuits significantly. In another study, Caleja et al. [67] found that enriching yogurts with natural and synthetic antioxidants did not change pH and nutritional values of yogurts samples significantly. Similarly, Fernandes et al. [68] investigated the sensory quality of lamb burgers prepared with the most promising natural antioxidants replacing sodium erythorbate. Their study revealed that natural antioxidants produced from spices have the potential to prevent changes that cause the deterioration of meat products. In the

same line, Taghvaei & Jafari [69] stated that several NFAs (caffeic acid, catechin, and myricetin) have high thermal stability and better antioxidant activity than SFAs in various edible oils. Finally, Yang et al. [70] stated that adding natural, plant-based (rosemary extract) antioxidants to vegetable oils prevents their lipid oxidation.

Regarding consumer attitudes, Mitterer-Daltoé et al. [71] investigated the consumers' cognitive assessment of food antioxidants by the qualitative consumer method Word Association. The results of the study demonstrated that consumers have a positive perception of natural antioxidants. In the same line, Rather et al. [72] found that consumers are increasingly choosing meat products with NFAs due to concerns about the adverse health effects of SFAs, particularly some synthetic antioxidants. Recently, Lungu et al. [73] surveyed awareness of antioxidants and attitudes towards the use of natural antioxidants as preservatives in meat and meat products. Their study revealed that participants supported use of natural antioxidants in these food products.

12.4.2 Consumers' Perceptions of Natural Colorants

Color has an important role in the acceptability and palatability of foods [75]. Colorants are used in many processed foods and beverage products, which play the role of compensating for coloration caused by exposure to moisture, light, air, processing, and storage conditions, have properties that include improving sensory aspects, correcting color variations, and providing food diversification [76]. Apart from the traditional use of FAs, food colorants have received special attention due to their ability to color foods strongly, as well as providing health benefits [77, 78]. Although food colorants have an insignificant weight share of the consumed products, and they have a large impact on consumers' perception of foods [79]. Furthermore, they are significant and controversial ingredients. Many studies were performed to learn about consumers' perceptions of the colors of food and beverages [80]. Table 12.5 lists the natural color additives approved by the FDA (Food and Drug Administration) [81].

Despite the technical advantages provided by synthetic colors, consumer demands for natural colorants are increasing. Compared to artificial colors and sweeteners, consumers perceive significantly more risks associated with colors [7]. Natural colorants, which provide health benefits and important technological and sensory properties to food systems, have emerged as an alternative to synthetic colorants because of consumers' concerns [82, 83]. Natural colorants are widely used in food systems [84–92]. However, because of technological issues, the use of natural colorants in food systems is still limited. Recently, Murley & Chambers [93] found that products derived from plants and products with natural colors and flavors were perceived to be the most natural. Consumers often associated the colors of beverages from natural sources with fruits, such as yellow for lemon or red for strawberry. Fruits are a great source of natural compounds, that allow the obtainment of a broad range of colorant molecules including carotenoids, anthocyanins, and

Table 12.5 Color additives approved by the FDA for use in human foods

Straight color	Uses and restrictions
Beet juice or powder	GMP
Annatto extract	GMP
Astaxanthin	Salmonid fish food only
Carrot oil	GMP
β-Apo-8′-carotenal	Foods and feeds
Caramel	GMP
Algae meal, dried	Chicken feed only
Corn endosperm oil	Chicken feed only
Tagetes (Aztec marigold) meal and extract	Chicken feed only
Carmine or cochineal extract	GMP
Canthaxanthin	Foods, salmonid fish feed, broiler chicken feed
Vegetable juice	GMP
Copper chlorophyllin, sodic	Citrus-based dry beverage mixes
Grape color extract	Non-beverage food only
Turmeric and turmeric oleoresin	GMP
Fruit juice	GMP
Paprika and paprika oleoresin	GMP
Grape skin extract (enocianina)	Beverages and beverage bases
Xanthophyllomyces dendrorhous (*Phaffia*) yeast	Salmonid fish feed only
Paracoccus pigment	Salmonid fish feed only
Cottonseed flour, toasted partially defatted cooked	GMP
Riboflavin	GMP
β-Carotene, natural and synthetic	GMP
Saffron	GMP
Haematococcus algae meal	Salmonid fish feed only
Ferrous gluconate or lactate	Ripe olives

FDA Food and Drug Administration; *GMP* Good Manufacturing Practices

chlorophylls [94]. Tennant & Klingenberg [95] found that exposures from food color use and coloring foods separately or combined are lower than those from a natural occurrence in foods. Nevertheless, consumers are very concerned about synthetic colorants.

12.4.3 Consumers' Perceptions of Natural Antimicrobials

Antimicrobials, also known as preservatives, are used to control the microbial growth in foods. These additives have been used for centuries in a variety of foods, along with other factors such as low storage temperatures, good sanitation practices and, application of physical microbial reduction treatments [96]. Due to the negative public perception of industrially synthesized food antimicrobials, the trend

towards the production and use of natural antimicrobials has increased [97]. Natural antimicrobials found in extracts from essential oils, herbs and spices, and other secondary metabolites from bacteria, herbs and enzymes have traditionally been of particular importance and are still widely used [98]. Besides acting as antimicrobials and antioxidants, natural compounds in plants also enhance color and flavor of food products [99]. Due to increasing consumers' demand for food free of synthetic preservatives, the demand for natural antimicrobial agents increases steadily for replacing synthetic compounds [100]. Hence, it is important to seek new sources of antimicrobial substances, including plant metabolites [101]. Tayengwa et al. [102] investigated the possibilities of dietary citrus pulp and grape pomace as natural preservatives for extending beef shelf life. These authors concluded that beef added of dried citrus pulp and dried grape pomace had lower coliform loads, as well as lipid and protein oxidation than control beef.

Regarding consumer perception, Perito et al. [6] investigated consumers' acceptance of food preservatives. Their results revealed that 64% of respondents stated that they would be willing to consume biological preservatives only if natural preservatives were used instead of synthetic preservatives. Finally, Hung et al. [103] investigated consumer attitude and purchase intention towards processed meat products with natural compounds and a reduced level of nitrite. The results of this study revealed that the use of natural additives against chemical additives positively affects consumers' purchase intention.

12.4.4 Consumers' Perceptions of Natural Sweeteners

Sweeteners have been used for decades to make food tastier and attract consumers. The preference for sweet taste is simply an innate universal attitude [104]. Sweetening agents are used to prepare chocolates, jams, sweets, beverages, soft-drinks, ice-creams, chewing-gums, candies, cakes, juices, and many other food items [105]. Since consumption of foods with low or no sugars increases, the food industries try to include sugar substitutes in food formulations [106, 107]. The physicochemical stability and sweetness profile during processing are the main factors for successful application of an alternative sweetener in a food [108]. Synthetic sweeteners and natural sweeteners have the same purpose of pretending to be sweet while dieting has fewer or no calories [109]. Since consumers pay more attention to their health, the demand for zero-calorie and naturally derived sweeteners has dramatically grown in the last decade [110]. Natural sweeteners contain a wide variety of compounds including amino acids, proteins, sugars, sugar alcohols, terpenoid glycosides, and some polyphenols [111]. Sylvetsky et al. [112] found that parents generally do not perceive synthetic sweeteners as safe for their children and frequently do not recognize common containing synthetic sweeteners foods and beverages. Farhat et al. [113] investigated the perceptions and knowledge of artificial sweeteners within the UK adult population. The results of their study revealed that risk perceptions are higher in women, older adults, and those with an education

qualification. Rusek et al. [114] investigated consumer knowledge and opinion on selected sweeteners. Their results revealed that 53% of respondents stated that sweeteners had a negative effect on health, and only 55% of responders think that legally approved sweeteners were safe. Therefore, consumers do not believe in all the information from different sources about sweeteners, but they rely on their own opinion.

12.4.5 Consumers' Perceptions of Texture Modifiers

Emulsifiers, stabilizers, and thickeners are widely used in foods as texturing modifiers [115]. The main aim of these texture modifiers is to maintain consistent texture and to prevent the separation of ingredients in such products as low-fat spreads, margarine, salad dressings, ice cream, and mayonnaise. Food emulsifiers are amphiphilic molecules that help create a mixture of oil and water. They are generally used for bakery products such as cakes, bread, and cookies. Thickeners contribute to increase the viscosity of foods [116]. They are added to foods such as salad dressings and flavored milk. The most common stabilizers used in milk products are listed in Table 12.6.

Demand for synthetic food emulsifiers as a food additive may decrease due to adverse health effects. New thickeners of natural origin derived from food biopolymers have always received significantly increased attention, especially in food fields where the edible safety of thickeners is highly demanded [125]. Varela and Fiszman [24] investigated consumers' knowledge and awareness of hydrocolloids used as food additives and food ingredients. As a result of their study, it was found that consumers had little knowledge and a relatively negative perception of the additives, but when consumers thought of additives, they did not have food hydrocolloids in mind.

Table 12.6 Use of food stabilizers in milk products

Stabilizer	Food matrix	Purpose	Reference
Guar gum	Yoghurt	Firmness	[117]
κ-Carrageenan	Vanilla ice cream	Reduction of hardness and iciness	[118]
Gelatin	Corn–milk yoghurt	Firmness	[119]
Locust bean gum	Ice cream	Viscousness	[120]
Xanthan	Yoghurt	Firmness	[121]
β-Lactoglobulin	Dressings	Creaminess	[122]
Pectin	Yoghurt	Compactness	[121]
Exopolysaccharides	Yoghurt, cheese, fermented cream milk based desserts	Firmness and creaminess	[123]

Adapted from Verma et al. [124]

12.5 Conclusions

The challenge for the industry is to work hard to inform and educate consumers about the safety of food additives and the role they play in delivering a wide range of fresh, nutritious, desired food with extended shelf life. The commercial future of any food additive is determined by consumer perceptions and resulting actions. Scaring people about additives and ingredients that are safe to use could well be one of the biggest risks to innovation in the sector. Surveys show that concern among consumers is steadily declining over time, but there is still a great deal of work to be done. Consumers are becoming more conscious and involved in what they eat, and both consumers and the food industry are showing more interest in natural foods. Consumers perceive naturalness as a beneficial property of foodstuffs. It is possible that these food products, which are not perceived as natural, are not accepted by a large number of consumers in most countries. Education may be required to develop knowledge and attitude about food additives. This chapter proposes more communication programs on food safety issues.

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References

1. Knorr D, Froehling A, Jaeger H, Reineke K, Schlueter O, Schoessler K. Emerging technologies in food processing. Annu Rev Food Sci Technol. 2011;2:203–35.
2. Khodjaeva U, Bojňanská T, Vietoris V, Sytar O. About food additives as important part of functional food. J Microbiol Biotechnol Food Sci. 2021;2021:2227–37.
3. Laganà P, Avventuroso E, Romano G, Gioffré ME, Patanè P, Parisi S, Moscato U, Delia S. Classification and technological purposes of food additives: the European point of view. In: Chemistry and hygiene of food additives. Cham: Springer; 2017. p. 1–21.
4. Kang HJ, Kim S, Lee G, Lim HS, Yun SS, Kim JW. Perception gaps on food additives among various groups in Korea: food experts, teachers, nutrition teachers, nongovernmental organization members, and general consumers. J Food Prot. 2017;80(6):1015–21.
5. Dickson-Spillmann M, Siegrist M, Keller C. Attitudes toward chemicals are associated with preference for natural food. Food Qual Prefer. 2011;22(1):149–56.
6. Perito MA, Chiodo E, Serio A, Paparella A, Fantini A. Factors influencing consumers' attitude towards biopreservatives. Sustainability. 2020;12(24):10338.
7. Bearth A, Cousin ME, Siegrist M. The consumer's perception of artificial food additives: influences on acceptance, risk and benefit perceptions. Food Qual Prefer. 2014;38:14–23.
8. Miao P, Chen S, Li J, Xie X. Decreasing consumers' risk perception of food additives by knowledge enhancement in China. Food Qual Prefer. 2020;79:103781.
9. Badora A, Bawolska K, Kozłowska-Strawska J, Domańska J. Food additives in food products: a case study. In: Nutrition in health and disease-our challenges now and forthcoming time. IntechOpen; 2019.

10. Silva MM, Lidon FC. An overview on applications and side effects of antioxidant food additives. Emirates J Food Agric. 2016;26(12):823–32.
11. Güngörmüş C, Kılıç A. The safety assessment of food additives by reproductive and developmental toxicity studies. Food Addit, InTech. 2012(1):31–48.
12. Zhong Y, Wu L, Chen X, Huang Z, Hu W. Effects of food-additive-information on consumers' willingness to accept food with additives. Int J Environ Res Public Health. 2018;15(11):2394.
13. Emerton V, Choi E. Essential guide to food additives, vol. 3. Cambridge, UK: Leatherhead Publishing; 2008.
14. Bolek S. Consumer knowledge, attitudes, and judgments about food safety: a consumer analysis. Trends Food Sci Technol. 2020;102:242–8.
15. Wu L, Zhang Q, Shan L, Chen Z. Identifying critical factors influencing the use of additives by food enterprises in China. Food Control. 2013;31(2):425–32.
16. Degreef F. "What's the deal with these strange substances in our food?" the representation of food additives by Belgian consumer organizations, 1960–1995. Food Foodways. 2019;27(1–2):144–63.
17. Tarnavölgyi G. Analysis of consumers' attitudes towards food additives using focus group survey. Agric Conspectus Scintificus. 2003;68(3):193–6.
18. Esfahani NB, Ziaei H, Esfandiari Z. The knowledge, attitudes, and practices toward food additives in personnel of Isfahan University of Medical Sciences in Iran. J Nutr Food Secur. 2021;6(2):161–9
19. Ismail BB, Fuchs R, Mohammad SF. Consumer awareness of the use of additives in processed foods. Ann Food Sci Technol. 2017;18(2):316–23.
20. Sachithananthan V. A study on the consumer awareness of food additives in packaged food and their effects on health in Abha region, Saudi Arabia. J Food Technol Preserv. 2017;1(3):14–27.
21. Hansen J, Holm L, Frewer L, Robinson P, Sandøe P. Beyond the knowledge deficit: Recent research into lay and expert attitudes to food risks. Appetite. 2003;41(2):111–21.
22. Carrillo E, Varela P, Salvador A, Fiszman S. Main factors underlying consumers' food choice: a first step for the understanding of attitudes toward "Healthy Eating". J Sens Stud. 2011;26(2):85–95.
23. Rembischevski P, Caldas ED. Risk perception related to food. Food Sci Technol (Campinas). 2020;2061:1–7.
24. Varela P, Fiszman SM. Exploring consumers' knowledge and perceptions of hydrocolloids used as food additives and ingredients. Food Hydrocoll. 2013;30(1):477–84.
25. Wongprawmas R, Canavari M. Consumers' willingnessto-pay for food safety labels in an emerging market: the case of fresh produce in Thailand. Food Policy. 2017;69:25–34.
26. Buchler S, Smith K, Lawrence G. Food risks, old and new: demographic characteristics and perceptions of food additives, regulation and contamination in Australia. J Sociol. 2010;46(4):353–74.
27. Wandel M, Fagerli RA. Consumer concern about food related health risks and their trust in experts. Ecol Food Nutr. 2001;40(3):253–83.
28. Eiser JR, Coulson NS, Eiser C. Adolescents' perceptions of the costs and benefits of food additives and their presence in different foods. J Risk Res. 2002;5(2):167–76.
29. Saraiva A, Carrascosa C, Raheem D, Ramos F, Raposo A. Natural sweeteners: the relevance of food naturalness for consumers, food security aspects, sustainability and health impacts. Int J Environ Res Public Health. 2020;17(17):6285.
30. Devcich DA, Pedersen IK, Petrie KJ. You eat what you are: modern health worries and the acceptance of natural and synthetic additives in functional foods. Appetite. 2007;48(3):333–7.
31. Rozin P, Fischler C, Shields-Argelès C. European and American perspectives on the meaning of natural. Appetite. 2012;59(2):448–55.
32. Bearth A, Cousin ME, Siegrist M. "The dose makes the poison": informing consumers about the scientific risk assessment of food additives. Risk Anal. 2016;36(1):130–44.

33. Gherezgihier BA, Mahmud A, Admassu H, Shui XW, Fang Y, Tsighe N, Mohammed JK. Food additives: functions, effects, regulations, approval and safety evaluation. J Acad Indus Res. 2017;6:62–8.
34. EFSA. Guidance for submission of food additives evaluations. EFSA J. 2012;10:2760.
35. Szűcs V, Szabó E, Guerrero L, Tarcea M, Bánáti D. Modelling of avoidance of food additives: a cross country study. Int J Food Sci Nutr. 2019;70(8):1020–32.
36. Magnuson B, Munro I, Abbot P, Baldwin N, Lopez-Garcia R, Ly K, McGirr L, Roberts A, Socolovsky S. Review of the regulation and safety assessment of food substances in various countries and jurisdictions. Food Addit Contamin Part A. 2013;30(7):1147–220.
37. Lorenzoni ASG, Oliveira FA, Cladera-Oliveira F. Food additives in products for children marketed in Brazil. Food Public Health. 2012;2(5):131–6.
38. Lockie S, Lyons K, Lawrence G, Grice J. Choosing organics: a path analysis of factors underlying the selection of organic food among Australian consumers. Appetite. 2004;43(2):135–46.
39. Rozin P. The meaning of "natural" process more important than content. Psychol Sci. 2005;16(8):652–8.
40. Siipi H. Is natural food healthy? J Agric Environ Ethics. 2013;26(4):797–812.
41. Carocho M, Barreiro MF, Morales P, Ferreira IC. Adding molecules to food, pros and cons: a review on synthetic and natural food additives. Compr Rev Food Sci Food Saf. 2014;13(4):377–99.
42. Santos-Sánchez NF, Salas-Coronado R, Valadez-Blanco R, Hernández-Carlos B, Guadarrama-Mendoza PC. Natural antioxidant extracts as food preservatives. Acta Sci Pol Technol Aliment. 2017;16(4):361–70.
43. Renn O. Risk communication–consumers between information and irritation. J Risk Res. 2006;9(8):833–49.
44. Rozin P, Fischler C, Shields-Argelès C. Additivity dominance: additives are more potent and more often lexicalized across languages than are``subtractives". Judgm Decis Mak. 2009;4(6):475.
45. Scotter MJ. Emerging and persistent issues with artificial food colours: natural colour additives as alternatives to synthetic colours in food and drink. Qual Assur Safety Crops Foods. 2011;3(1):28–39.
46. Grunert KG. Food quality and safety: consumer perception and demand. Eur Rev Agric Econ. 2005;32(3):369–91.
47. Rozin P. Naturalness judgments by lay Americans: process dominates content in judgments of food or water acceptability and naturalness. Judgm Decis Mak. 2006;1(2):91.
48. Rozin P, Spranca M, Krieger Z, Neuhaus R, Surillo D, Swerdlin A, Wood K. Preference for natural: instrumental and ideational/moral motivations, and the contrast between foods and medicines. Appetite. 2004;43(2):147–54.
49. Siegrist M, Sütterlin B. Importance of perceived naturalness for acceptance of food additives and cultured meat. Appetite. 2017;113:320–6.
50. Siegrist M. The influence of trust and perceptions of risks and benefits on the acceptance of gene technology. Risk Anal. 2000;20(2):195–204.
51. European Commission. Special Eurobarometer: attitudes of European citizens towards the environment, 2004
52. Dosman DM, Adamowicz WL, Hrudey SE. Socioeconomic determinants of health-and food safety-related risk perceptions. Risk Anal. 2001;21(2):307–18.
53. Knight A, Warland R. The relationship between sociodemographics and concern about food safety issues. J Consum Aff. 2004;38(1):107–20.
54. Roininen K, Lähteenmäki L, Tuorila H. Quantification of consumer attitudes to health and hedonic characteristics of foods. Appetite. 1999;33(1):71–88.
55. Dickson-Spillmann M, Siegrist M, Keller C, Wormuth M. Phthalate exposure through food and consumers' risk perception of chemicals in food. Risk Anal Int J. 2009;29(8):1170–81.
56. Schifferstein HN, Ophuis PAO. Health-related determinants of organic food consumption in the Netherlands. Food Qual Prefer. 1998;9(3):119–33.

57. Quinchia LA, Delgado MA, Valencia C, Franco JM, Gallegos C. Natural and synthetic antioxidant additives for improving the performance of new biolubricant formulations. J Agric Food Chem. 2011;59(24):12917–24.
58. Cömert ED, Gökmen V. Evolution of food antioxidants as a core topic of food science for a century. Food Res Int. 2018;105:76–93.
59. Lourenço SC, Moldão-Martins M, Alves VD. Antioxidants of natural plant origins: from sources to food industry applications. Molecules. 2019;24(22):4132.
60. Saad B, Sing YY, Nawi MA, Hashim N, Ali ASM, Saleh MI, Sulaiman SF, Talib K, Ali ASM. Determination of synthetic phenolic antioxidants in food items using reversed-phase HPLC. Food Chem. 2007;105(1):389–94.
61. Gutiérrez-del-Río I, López-Ibáñez S, Magadán-Corpas P, Fernández-Calleja L, Pérez-Valero Á, Tuñón-Granda M, Miguélez EM, Villar CJ, Lombó F. Terpenoids and polyphenols as natural antioxidant agents in food preservation. Antioxidants. 2021;10(8):1264.
62. Atta EM, Mohamed NH, Silaev AAA. Antioxidants: an overview on the natural and synthetic types. Eur Chem Bullet. 2017;6(8):365–75.
63. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004;74(17):2157–84.
64. Li Y, Zhang JJ, Xu DP, Zhou T, Zhou Y, Li S, Li HB. Bioactivities and health benefits of wild fruits. Int J Mol Sci. 2016;17(8):1258.
65. Zhang JJ, Li Y, Zhou T, Xu DP, Zhang P, Li S, Li HB. Bioactivities and health benefits of mushrooms mainly from China. Molecules. 2016;21(7):938.
66. Caleja C, Barros L, Antonio AL, Oliveira MBP, Ferreira IC. A comparative study between natural and synthetic antioxidants: evaluation of their performance after incorporation into biscuits. Food Chem. 2017;216:342–6.
67. Caleja C, Barros L, Antonio AL, Carocho M, Oliveira MBP, Ferreira IC. Fortification of yogurts with different antioxidant preservatives: a comparative study between natural and synthetic additives. Food Chem. 2016;210:262–8.
68. Fernandes RDP, Trindade MA, Tonin FG, Lima CG, Pugine SMP, Munekata PES, Lorenzo JM, De Melo MP. Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. J Food Sci Technol. 2016;53(1):451–60.
69. Taghvaei M, Jafari SM. Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. J Food Sci Technol. 2015;52(3):1272–82.
70. Yang Y, Song X, Sui X, Qi B, Wang Z, Li Y, Jiang L. Rosemary extract can be used as a synthetic antioxidant to improve vegetable oil oxidative stability. Ind Crop Prod. 2016;80:141–7.
71. Mitterer-Daltoé M, Bordim J, Lise C, Breda L, Casagrande M, Lima V. Consumer awareness of food antioxidants. Synthetic vs. Natural. Food Sci Technol. 2020;41:208–12
72. Rather SA, Masoodi FA, Akhter R, Rather JA, Shiekh KA. Advances in use of natural antioxidants as food additives for improving the oxidative stability of meat products. Madridge J Food Technol. 2016;1(1):10–7.
73. Lungu NS, Afolayan AJ, Thomas RS, Idamokoro EM. Consumer exposure to warmed-over flavour and their attitudes towards the use of natural antioxidants as preservatives in meat and meat products. Br Food J. 2020;122(9):2927–2937.
74. Sarkar A, Ghosh U. Natural antioxidants-the key to safe and sustainable life. Int J Latest Trends Eng Technol. 2016;6(3):460–6.
75. Piqueras-Fiszman B, Spence C. Sensory expectations based on product-extrinsic food cues: an interdisciplinary review of the empirical evidence and theoretical accounts. Food Qual Prefer. 2015;40:165–79.
76. Martins FCOL, Sentanin MA, Souza D. Analytical methods in food additives determination: compounds with functional applications. Food Chem. 2019;272:732–50.
77. Gengatharan A, Dykes GA, Choo WS. Betalains: natural plant pigments with potential application in functional foods. LWT-Food Sci Technol. 2015;64(2):645–9.

78. Shim SM, Seo SH, Lee Y, Moon GI, Kim MS, Park JH. Consumers' knowledge and safety perceptions of food additives: evaluation on the effectiveness of transmitting information on preservatives. Food Control. 2011;22(7):1054–60.
79. Gebhardt B, Sperl R, Carle R, Müller-Maatsch J. Assessing the sustainability of natural and artificial food colorants. J Clean Prod. 2020;260:120884.
80. Spence C, Levitan CA, Shankar MU, Zampini M. Does food color influence taste and flavor perception in humans? Chemosens Percept. 2010;3(1):68–84.
81. FDA. Color additives inventories. 2012. http://www.fda.gov. Accessed 14 Oct 2021.
82. Luzardo-Ocampo I, Ramírez-Jiménez AK, Yañez J, Mojica L, Luna-Vital DA. Technological applications of natural colorants in food systems: a review. Foods. 2021;10(3):634.
83. Rodriguez-Amaya DB. Natural food pigments and colorants. Curr Opin Food Sci. 2016;7:20–6.
84. Abdel-Moemin AR. Effect of Roselle calyces extract on the chemical and sensory properties of functional cupcakes. Food Sci Human Wellness. 2016;5(4):230–7.
85. Amjadi S, Ghorbani M, Hamishehkar H, Roufegarinejad L. Improvement in the stability of betanin by liposomal nanocarriers: its application in gummy candy as a food model. Food Chem. 2018;256:156–62.
86. Carballo DE, Caro I, Andrés S, Giráldez FJ, Mateo J. Assessment of the antioxidant effect of astaxanthin in fresh, frozen and cooked lamb patties. Food Res Int. 2018;111:342–50.
87. Da Silva LP, Pereira E, Prieto MA, Simal-Gandara J, Pires TC, Alves MJ, Calhelha R, Barros L, Ferreira IC. Rubus ulmifolius Schott as a novel source of food colorant: extraction optimization of coloring pigments and incorporation in a bakery product. Molecules. 2019;24(11):2181.
88. Freitas-Sá DDGC, de Souza RC, de Araujo MCP, Borguini RG, de Mattos LDS, Pacheco S, de Oliveira Godoy RL. Effect of jabuticaba (Myrciaria jaboticaba (Vell) O. Berg) and jamelão (Syzygium cumini (L.) Skeels) peel powders as colorants on color-flavor congruence and acceptability of yogurts. LWT. 2018;96:215–21.
89. Montoya-Rodríguez A, Milán-Carrillo J, Dia VP, Reyes-Moreno C, de Mejía EG. Pepsin-pancreatin protein hydrolysates from extruded amaranth inhibit markers of atherosclerosis in LPS-induced THP-1 macrophages-like human cells by reducing expression of proteins in LOX-1 signaling pathway. Proteome Sci. 2014;12(1):1–13.
90. López CJ, Caleja C, Prieto MA, Sokovic M, Calhelha RC, Barros L, Ferreira IC. Stability of a cyanidin-3-O-glucoside extract obtained from Arbutus unedo L. and incorporation into wafers for colouring purposes. Food Chem. 2019;275:426–38.
91. Rodríguez-Sánchez JA, Victoria MTC, Barragán-Huerta BE. Betaxanthins and antioxidant capacity in Stenocereus pruinosus: stability and use in food. Food Res Int. 2017;91:63–71.
92. Sharma P, Segat A, Kelly AL, Sheehan JJ. Colorants in cheese manufacture: production, chemistry, interactions, and regulation. Compr Rev Food Sci Food Saf. 2020;19(4):1220–42.
93. Murley T, Chambers E. The influence of colorants, flavorants and product identity on perceptions of naturalness. Foods. 2019;8(8):317.
94. Albuquerque BR, Oliveira MBP, Barros L, Ferreira IC. Could fruits be a reliable source of food colorants? Pros and cons of these natural additives. Crit Rev Food Sci Nutr. 2021;61(5):805–35.
95. Tennant DR, Klingenberg A. Consumer exposures to anthocyanins from colour additives, colouring foodstuffs and from natural occurrence in foods. Food Addit Contamin Part A. 2016;33(6):959–67.
96. Erickson MC, Doyle MP. The challenges of eliminating or substituting antimicrobial preservatives in foods. Annu Rev Food Sci Technol. 2017;8:371–90.
97. Faleiro ML. The mode of antibacterial action of essential oils. In: Science against microbial pathogens: communicating current research and technological advances, vol. 2; 2011. p. 1143–56.
98. Batiha GES, Hussein DE, Algammal AM, George TT, Jeandet P, Al-Snafi AE, Tiwari A, Pagnossa JP, Lima CM, Thorat ND, Zahoor M, El-Esawi M, Dey A, Alghamdi S, Hetta HF,

Cruz-Martins N. Application of natural antimicrobials in food preservation: recent views. Food Control. 2021;126:108066.
99. Corbo MR, Bevilacqua A, Campaniello D, D'Amato D, Speranza B, Sinigaglia M. Prolonging microbial shelf life of foods through the use of natural compounds and non-thermal approaches–a review. Int J Food Sci Technol. 2009;44(2):223–41.
100. Quinto EJ, Caro I, Villalobos-Delgado LH, Mateo J, De-Mateo-Silleras B, Redondo-Del-Río MP. Food safety through natural antimicrobials. Antibiotics. 2019;8(4):208.
101. Del Nobile MA, Lucera A, Costa C, Conte A. Food applications of natural antimicrobial compounds. Front Microbiol. 2012;3:287.
102. Tayengwa T, Chikwanha OC, Gouws P, Dugan ME, Mutsvangwa T, Mapiye C. Dietary citrus pulp and grape pomace as potential natural preservatives for extending beef shelf life. Meat Sci. 2020;162:108029.
103. Hung Y, de Kok TM, Verbeke W. Consumer attitude and purchase intention towards processed meat products with natural compounds and a reduced level of nitrite. Meat Sci. 2016;121:119–26.
104. Drewnowski A, Mennella JA, Johnson SL, Bellisle F. Sweetness and food preference. J Nutr. 2012;142(6):1142S–8S.
105. Priya K, Gupta VRM, Srikanth K. Natural sweeteners: a complete review. J Pharm Res. 2011;4(7):2034–9.
106. Costa GM, Paula MM, Barão CE, Klososki SJ, Bonafé EG, Visentainer JV, Cruz AG, Pimentel TC. Yoghurt added with Lactobacillus casei and sweetened with natural sweeteners and/or prebiotics: implications on quality parameters and probiotic survival. Int Dairy J. 2019;97:139–48.
107. Gao J, Brennan MA, Mason SL, Brennan CS. Effects of sugar substitution with "Stevianna" on the sensory characteristics of muffins. J Food Qual. 2017;2017:1–11.
108. Lee SH, Choe SY, Seo GG, Hong JH. Can "Functional Sweetener" context increase liking for cookies formulated with alternative sweeteners? Foods. 2021;10(2):361.
109. Neacsu NA, Madar A. Artificial sweeteners versus natural sweeteners. Bulletin of the Transilvania University of Brasov. Econ Sci Ser V. 2014;7(1):59.
110. Philippe RN, De Mey M, Anderson J, Ajikumar PK. Biotechnological production of natural zero-calorie sweeteners. Curr Opin Biotechnol. 2014;26:155–61.
111. Chéron J-B, Marchal A, Fiorucci S. Natural sweeteners. In: Varelis P, Melton L, Shahidi F, editors. Encyclopedia of food chemistry, vol. 1. Amesterdam: Elsevier; 2019. p. 189–195.
112. Sylvetsky AC, Greenberg M, Zhao X, Rother KI. What parents think about giving nonnutritive sweeteners to their children: a pilot study. Int J Pediatr. 2014;2014:819872.
113. Farhat G, Dewison F, Stevenson L. Knowledge and perceptions of non-nutritive sweeteners within the UK adult population. Nutrients. 2021;13(2):444.
114. Rusek A, Biazik E, Lesiów T. Consumer knowledge and opinion on selected sweeteners used in food. Part 2. Nauki Inżynierskie i Technologie. 2017;1(24)
115. Halmos EP, Mack A, Gibson PR. emulsifiers in the food supply and implications for gastrointestinal disease. Aliment Pharmacol Ther. 2019;49(1):41–50.
116. Kim CY, Yoo B. Rheological characterization of thickened protein-based beverages under different food thickeners and setting times. J Texture Stud. 2018;49(3):293–9.
117. Hassan LK, Haggag HF, ElKalyoubi MH, Abd EL-Aziz M, El-Sayed MM, Sayed AF. Physicochemical properties of yoghurt containing cress seed mucilage or guar gum. Ann Agric Sci. 2015;60(1):21–8.
118. BahramParvar M, Tehrani MM, Razavi SM. Effects of a novel stabilizer blend and presence of κ-carrageenan on some properties of vanilla ice cream during storage. Food Biosci. 2013;3:10–8.
119. Supavititpatana P, Wirjantoro TI, Apichartsrangkoon A, Raviyan P. Addition of gelatin enhanced gelation of corn–milk yogurt. Food Chem. 2008;106(1):211–6.
120. Barak S, Mudgil D. Locust bean gum: processing, properties and food applications—a review. Int J Biol Macromol. 2014;66:74–80.

121. Everett DW, McLeod RE. Interactions of polysaccharide stabilisers with casein aggregates in stirred skim-milk yoghurt. Int Dairy J. 2005;15(11):1175–83.
122. Christiansen KF, Vegarud G, Langsrud T, Ellekjaer MR, Egelandsdal B. Hydrolyzed whey proteins as emulsifiers and stabilizers in high-pressure processed dressings. Food Hydrocoll. 2004;18(5):757–67.
123. Duboc P, Mollet B. Applications of exopolysaccharides in the dairy industry. Int Dairy J. 2001;11(9):759–68.
124. Verma DK, Kumar Mahato D, Billoria S, Kapri M, Prabhakar PK, Kumar A, Srivastav PP. Microbial Spoilage in Milk and Milk products: potential solution, food safety, and health issues. In: Microorganisms in sustainable agriculture, food, and the environment. Apple Academic Press; 2017. p. 171–95.
125. Wei Y, Guo Y, Li R, Ma A, Zhang H. Rheological characterization of polysaccharide thickeners oriented for dysphagia management: Carboxymethylated curdlan, konjac glucomannan and their mixtures compared to xanthan gum. Food Hydrocoll. 2021;110:106198.

Chapter 13
Regulation of Natural Food Additives

Sebahat Öztekin, Katya Anaya, and Aysun Yurdunuseven-Yıldız

13.1 Introduction

Food additives (FAs) are intentionally added to foods to improve the safety, texture, appearance, freshness, and flavor after being subjected to a thorough scientific safety examination by the relevant authorities [1]. In this context, Codex Alimentarius Commission (CAC) establishes maximum levels of FAs deemed safe by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [2, 3]. The regulations on FAs may vary across the countries for instance, in the United States, Food and Drug Administration (FDA) [4]; in Europe, European Food Safety Authority (EFSA) [5]; in Japan, Ministry of Health, Labor and Welfare (MHLW) [6]; in India, Food Safety and Standards Authority of India (FSSAI) [7]; and in Argentina, Brazil, Paraguay, and Uruguay, Mercosur [8] approve the FAs. All of the regulations aim to ensure the safety of FAs and define requirements for their continued use in food. However, the allowable concentrations and carry-over of FAs, authorization procedures, labelling, processing aids, and accessibility may differ among them [9].

FAs can be divided into four basic groups based on their origin and manufacture: natural food additives (NFAs), obtained directly from animals or plants; similar to NFAs, modified from natural, and synthetic food additives (SFAs) [10].

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Naturalness in food products has captivated consumers and policymakers due to its health and environmental benefits. In this sense, NFAs are increasingly used in functional foods inventing the term "clean label" with their health-promoting properties [11, 12]. The shift to natural foods has been led by Europe, with the goal of replacing SFAs with NFAs. However, there is no universal or clear definition of "natural," and substantial clinical trials are required to receive approval for a health claim. Furthermore, because totally natural alternatives do not yet exist technically or commercially, our food's shift to full naturalness was delayed. There is no regulatory definition of the phrase "natural" for any elements added to food, with the exception of flavourings, especially from the consumer's standpoint. To date, the most thorough definition of "natural" has been given in Annex II (Article 16.2) of Regulation (EC) No. 1334/2008 on flavourings; "*The term 'natural' for the description of a flavouring may only be used if the flavouring component comprises only flavouring preparations and/or natural flavouring substances"*. Among six categories of flavourings, two groups are considered in the natural status: flavouring substances and flavouring preparations. The European authorities aim to draft a NFAs legislation within the European regulatory framework, based on the concept of natural in the flavourings regulations. However, foods that were previously deemed natural may no longer be considered natural if the flavourings legislation is applied to them. Thermally processed flavouring is not considered natural in the EU, despite being made entirely of natural materials. According to flavourings legislation, canned foods, breakfast cereals, and snack foods cannot be considered as natural status due to excessive heat application (>120 °C) during their processing [13]. Apart from this, "natural" definitions are available in the natural flavourings in the US (21CFR101.22) especially for meat products [14]. More recently, technical specifications (TS) 19657 on NFAs was published in 2017, ensuring a "level-playing field and fair practices in business-to-business relationships" [15]. However, TS 19657 provides a correct but inadequate interpretation of customers' views of the naturalness of food ingredients [16].

In Europe, all approved FAs (natural or synthetic) have an E-number, where E stands for Europe. The E-numbering system was developed to assist consumers in determining which additives have been approved for usage in the European Union [17]. Although clean labelled products contain E-numbered ingredients which are hidden by using their full name on the label, generating a natural consumer perception. For example, instead of showing E392 on the food label, "extract of rosemary" can be written, which may unjustifiably infer that food is of higher quality than its counterparts. To emphasize the naturalness, some food products are intentionally labelled with "containing only natural colours" although there is no proper definition of natural colours. However, some consumers believe that FAs reduce the integrity and purity of the product; this is mainly the case if the additive has an E number. Therefore, a universally accepted definition of "natural" is highly needed along with their regulations within the scope of food ingredients, additives, and flavourings [18].

Each food additive was investigated for possible toxicity to determine the NOAEL (no observed adverse effect level) and the ADI (acceptable daily intake), the amount of a food additive, expressed as mg/kg body weight. Before approval,

the maximum permitted levels need to be determined for each food group. *Quantum satis* was defined as "no maximum level is specified and substances shall be used following good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled" by EFSA in the Regulation (EC) No 1333/2008. In addition, Regulation (EC) No 1331/2008 [19] set a common authorization for FAs, enzymes, and flavorings. Based on the regulations above, only approved FAs can be commercialized in Europe. FAs can be re-evaluated if there is a potential risk to human health [20]. Currently, EFSA [21] re-evaluated colorants in 2015; preservatives, antioxidants, glutamates, silicon dioxide in 2016; all other additives except sweeteners in 2018; and finally, sweeteners in 2020 [22]. Development of new NFAs would be accelerated if there was a more uniform international approach to their approval with full toxicological evaluation [18].

The regulations for FAs mentioned above set the standards for their ongoing use in food while ensuring food safety and fair trade practices; however, there is no separate regulation for NFAs yet [23, 24]. In this line, natural colorants, natural antioxidants, natural antimicrobials (preservatives), and natural sweeteners were covered in this chapter, focusing on regulations in Europe, the United States, and other countries.

13.2 Regulation of Natural Food Additives in the European Union

The European Union (EU) maintains a stringent protocol for human consumption and international trade of FAs. The European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food (ANS) assesses FAs together with the Food Ingredient and Packaging (FIP) Unit [25]. The EU legal definition of a 'food additive' is defined in Regulation (EC) No 1333/2008 as meaning *"any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods"*. In this context, FAs should meet requirements that contain information to appropriately identify the food additive, including its origin and a description of the permissible purity requirements [26]. Firstly, European Commission (EC) proposed a common regulation for FAs, enzymes, and flavors with Regulation (EC) No 1331/2008. The following regulations (1332/2008 for enzymes, 1332/2008 for FAs, and 1334/2008 for flavourings) were adopted under the Food Improvement Agents Package (FIAP) [19]. All approved FAs in the EU must meet the specifications outlined in Regulation (EC) No 231/2012 established by Regulation (EC) No 1333/2008. Names, synonyms, chemical and physical properties, origin/

Table 13.1 Natural food additives used in the European Union (EU)

Additive class	Natural additive	Regulation	Group	E-number	Use in food/Foodstuffs in EU
Colorants	Curcumin	(EU) No 1129/2011	III	E 100	Meat, non-heat or heat-treated meat products, meat casings and coatings, processed potatoes, fruit and vegetable preparations/spreads, processed cheese, processed fish and fishery products (including molluscs and crustaceans), aromatized wine and wine-based drinks, fats, oils, jam, jellies and marmalades, and sweetened chestnut purée
	Riboflavins	(EU) No 1129/2011	II	E 101	Canned/bottled/dried fruit and vegetables, non-heat-treated processed meat, casings, and coatings and decorations for meat, processed fish and fisheries products including molluscs and crustaceans, aromatized wines and wine-based drinks, fruit and vegetables in vinegar, oil, or brine, fruit and vegetable preparations excluding compote, processed potato products
	Cochineal, carminic acid, carmines	(EU) No 1129/2011	III	E 120	Cheese (ripened, processed), cheese products, fruit and vegetables (dried; in vinegar, oil, or brine; canned or bottled; jam, jellies, and marmalades; spreads), breakfast cereal, meat preparations, heat- and non-heat-processed meat, meat casing and coatings, processed fish and fishery products (including molluscs and crustaceans), aromatized wine and wine-based drinks
	Chlorophylls and chlorophyllins	(EU) No 1129/2011	II	E 140	Ripened cheese, dried/canned/bottled fruit or vegetables, fruit and vegetable preparations excluding compote, jam, jellies, and marmalades and sweetened chestnut puree, processed fish and fisheries products (including molluscs and crustaceans), fruit and vegetables in vinegar, oil, or brine
	Copper complexes of chlorophylls and chlorophyllins	(EU) No 1129/2011	II	E 141	Dried/canned/bottled fruit or vegetables, fruit and vegetables in vinegar, oil, or brine, jam, jellies and marmalades and sweetened chestnut puree, processed fish and fishery products (including molluscs and crustaceans), ripened cheese, fruit and vegetable preparations excluding compote
	Plain caramel	(EU) No 0738/2013	II	E 150a	Fruit and vegetable preparations excluding compote

Additive class	Natural additive	Regulation	Group	E-number	Use in food/Foodstuffs in EU
	Carotenes	(EU) No 1129/2011	II	E 160a	Ripened/processed cheese and cheese products, other fat and oil emulsions (including spreads), fats and oils essentially free from water, butter and concentrated butter and butter oil and anhydrous milkfat, dried/canned/bottled fruit or vegetables, fruit, and vegetables in vinegar, oil, or brine, jam, jellies and marmalades, and sweetened chestnut puree, breakfast cereals, non-heat or heat-treated meat products, processed fish and fishery products (including molluscs and crustaceans), fruit and vegetable preparations excluding compote, processed potato products
	Annatto bixin	(EU) No 2020/0771	Ni	E 160b(i)	Flavored fermented milk products including heat-treated products, ripened /processed cheese, edible cheese rind, fats, and oils essentially free from water (excluding anhydrous milkfat), other fat and oil emulsions including spreads, jam, jellies and marmalades, and sweetened chestnut puree, other similar fruit or vegetable spreads, processed potato products, other confectionery including breath refreshening microsweets, decorations, coatings, and fillings, except fruit-based fillings, noodles, batters, meat, non-heat or heat-treated meat products, casings and coatings and decorations for meat, processed fish and fisheries products including molluscs and crustaceans, soups and broths, sauces, flavored and spirit drinks, potato-, cereal-, flour- or starch-based snacks, processed nuts, desserts excluding products
	Annatto norbixin	(EU) No 2020/0771	Ni	E 160b(ii)	Flavored fermented milk products including heat-treated products, ripened/edible/processed cheese and cheese products, edible ices, jam, jellies, and marmalades, and sweetened chestnut puree, other similar fruit or vegetable spreads, processed potato products, other confectionery, including breath refreshening microsweets, decorations, coatings, and fillings, except fruit-based fillings, breakfast cereals, noodles, batters, fine bakery wares, meat, non-heat or heat-treated meat products, meat casings and coatings, processed fish and fisheries products including molluscs and crustaceans, soups and broths, sauces, other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol, potato-, cereal-, flour- or starch-based snacks, processed nuts, desserts excluding products

(continued)

Table 13.1 (continued)

Additive class	Natural additive	Regulation	Group	E-number	Use in food/Foodstuffs in EU
	Paprika extract, capsanthin, capsorubin	(EU) No 1129/2011	II	E 160c	Processed /ripened cheese, cheese products, dried fruit and vegetables, fruit and vegetables in vinegar, oil, or brine, canned or bottled fruit and vegetables, jam, jellies and marmalades, and sweetened chestnut puree, breakfast cereals, non-heat or heat-treated meat products, processed fish and fisheries products (including molluscs and crustaceans), meat preparations, fruit and vegetable preparations excluding compote
	Lycopene	(EU) No 1129/2011	Ni	E 160d	Flavored fermented milk products including heat-treated products, other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol, food supplements supplied in a liquid form, (excluding food supplements for infants and young children), processed cheese, edible ices, jam, jellies and marmalades and sweetened chestnut puree, other similar fruit or vegetable spreads, other confectionery including breath refreshening microsweets, dietary foods for special medical purposes, dietary foods for weight control diets intended to replace total daily food intake or an individual meal, potato-, cereal-, flour- or starch-based snacks, processed nuts, food supplements supplied in a solid form (excluding food supplements for infants and young children), chewing gum, batters, fine bakery wares, casings and coatings and decorations for meat, fish roe, seasonings and condiments, soups and broths, sauces, protein products, flavored drinks, aromatised wine-product cocktails, decorations, coatings and fillings, except fruit-based fillings, edible cheese rind, fruit wine and made wine, processed fish and fisheries products (including molluscs and crustaceans)
	Lutein	(EU) No 0232/2012	III	E 161b	Processed cheese, jam, jellies and marmalades, sweetened chestnut puree, other similar fruit or vegetable spreads, processed fish and fisheries products (including molluscs and crustaceans)
	Beetroot red, betanin	(EU) No 1129/2011	II	E 162	Dried fruit and vegetables, fruit and vegetables in vinegar, oil, or brine, canned or bottled fruit and vegetables, jam, jellies and marmalades, and sweetened chestnut puree, breakfast cereals, meat preparations, non-heat or heat-treated processed meats, processed fish, and fisheries products (including molluscs and crustaceans), fruit and vegetable preparations excluding compote

Additive class	Natural additive	Regulation	Group	E-number	Use in food/Foodstuffs in EU
	Anthocyanins	(EU) No 1129/2011	II	E 163	Ripened cheese, cheese products, dried/canned/ bottled fruit and vegetables, fruit and vegetables in vinegar, oil, or brine, jam, jellies and marmalades, and sweetened chestnut puree, breakfast cereals, processed fish and fisheries products (including molluscs and crustaceans), fruit and vegetable preparations excluding compote, aromatised wines
Antioxidants	Ascorbic acid	(EU) No 1129/2011	I	E 300	Dehydrated milk, frozen/canned/bottled fruit and vegetables, compote, excluding products, extra jam, and extra jelly, jam, jellies and marmalades, and sweetened chestnut puree, other similar fruit or vegetable spreads, fresh and pre-cooked pasta, bread prepared solely with the following ingredients: wheat flour, water, yeast or leaven, salt, pain courant francais; friss búzakenyér, fehér és félbarna kenyerek, unprocessed fish, unprocessed molluscs and crustaceans, fruit juices and nectars, beer and malt beverages, heat-treated processed meat, processed cereal-based foods and baby foods for infants and young children, meat preparations, flours, fats and oils essentially free from water (excluding anhydrous milkfat), peeled, cut and shredded fruit and vegetables
	Tocopherol-rich extract	(EU) No 1129/2011	I	E 306	Fats and oils essentially free from water (excluding anhydrous milkfat), infant and follow-on formulae, processed cereal-based foods and baby foods for infants and young children, and other foods for young children
	Extracts of rosemary	(EU) No 1129/2011	Ni	E 392	Dehydrated milk, fats, and oils essentially free from water (excluding anhydrous milkfat), vegetable oil pan spray, chewing gum, processed eggs, and egg products, nut butters and nut spreads, fine bakery wares, seasonings and condiments, processed nuts, mustard, sauces, soups and broths, potato-, cereal-, flour- or starch-based snacks, food supplements supplied in a solid/liquid form, excluding food supplements for infants and young children, decorations, coatings, and fillings, except fruit-based fillings, fillings of stuffed pasta (ravioli and similar), processed fish and fisheries products (including molluscs and crustaceans), fruit and vegetable preparations excluding compote, processed potato products, non-heat or heat-treated processed meats, other fat and oil emulsions including spreads

(continued)

Table 13.1 (continued)

Additive class	Natural additive	Regulation	Group	E-number	Use in food/Foodstuffs in EU
Antimicrobials (preservatives)	Natamycin	(EU) No 1129/2011	Ni	E 235	Non-heat or heat-treated processed meats, ripened cheese and cheese products
	Nisin	(EU) No 1129/2011	Ni	E 234	Ripened/unripened/processed cheese and cheese products, other creams, processed eggs, and egg products, desserts
	Lysozyme	(EU) No 1129/2011	Ni	E 1105	Ripened cheese and cheese products, beer and malt beverages, fruit wine and made wine, other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol
Sweeteners	Thaumatin	(EU) No 1129/2011	Ni	E 957	Flavored fermented milk products including heat-treated products, edible ices, cocoa, and chocolate products, chewing gum, table-top sweeteners in liquid/powder/tablets form, flavored drinks, desserts, other confectionery including breath refreshening microsweets, decorations, coatings and fillings, sauces, potato-, cereal-, flour- or starch-based snacks, food supplements supplied in a solid/liquid form, excluding food supplements for infants and young children
	Erythritol	(EU) No 2015/1832	I and IV	E 968	Flavored drinks
	Steviol glycosides	(EU) 2021/1156	Ni	E 960	Flavored fermented milk products including heat-treated products, edible ices, fruit and vegetables in vinegar, oil, or brine, fruit and vegetable preparations excluding compote, cocoa and chocolate products, chewing gum, decorations, coatings and fillings, except fruit-based fillings, breakfast cereals, fine bakery wares, table-top sweeteners in liquid/powder/tablets form, soups and broths, sauces, dietary foods for special medical purposes, dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet), fruit nectars, beer and malt beverages, other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol, potato-, cereal-, flour- or starch-based snacks, processed nuts, desserts excluding products, extra jam and extra jelly, jam, jellies and marmalades and sweetened chestnut puree, other similar fruit or vegetable spreads, processed fish and fisheries products including molluscs and crustaceans, flavored drinks, mustard, other confectionery including breath refreshening microsweets, food supplements supplied in a solid/liquid form, excluding food supplements for infants and young children

Ni Not informed

source and process of preparation of the substance, the appearance of the substance, E numbers, conditions of use, the maximum levels of use, specific identification tests, and purity criteria are included for each additive [27]. Regulation (EC) No 1333/2008 contains the following five annexes:

- Annex I list the 27 EU functional classes of FAs, including sweeteners, colors, preservatives, antioxidants, carriers, acids, acidity regulators, anti-caking agents, anti-foaming agents, bulking agents, emulsifiers, emulsifying salts, firming agents, flavor enhancers, foaming agents, gelling agents, glazing agents, humectants, modified starches, packaging gases, propellants, raising agents, sequestrants, stabilizers, thickeners, flour treatment agents and contrast enhancers,
- Annex II lists a Union list of FAs approved for use in foods and their conditions of use,

Table 13.2 Natural Colorants Approved for use in Foods for Human Consumption According to the Food and Drug Administration from United States

Natural colorant	Color	Origin
Annatto extract	Yellow to orange-red	Plant
Beet powder	Bluish-red to brown	Plant
Beta-carotene	Yellow to orange	Plant, Algae, Fungi
Butterfly pea flower extract	Purple to denim blue	Plant
Calcium carbonate	White	Mineral
Canthaxanthin	Reddish-orange	Fungi, Algae, Bacteria[a]
Caramel	Yellow to brown, black	Plant
Carrot oil	Purple, orange	Plant
Cochineal extract; carmine	Red	Animal, Fungus[b]
Fruit or vegetable juices, concentrated or dried	Variable	Plant
Grape skin extract	Red, green	Plant
Mica-based pearlescent pigments	Glittery, metallic, silky – varying colors	Mineral
Paprika extract	Orange-red	Plant
Riboflavin	Yellow to orange-yellow	Bacteria[c]
Saffron	Yellow-orange	Plant
Soy leghemoglobin	Reddish-brown	Yeast
Spirulina extract (phycocyanin)	Blue	Algae
Toasted partially defatted cooked cottonseed flour	Brown	Plant
Tomato lycopene extract; tomato lycopene concentrate	Red	Plant
Turmeric, curcumin	Yellow	Plant

[a][59]
[b][58]
[c][60]

- Annex III lists a Union list of FAs, including carriers approved for use in FAs, food enzymes, food flavourings, nutrients, and their conditions of use,
- Annex IV lists traditional foods for which certain Member States may continue to prohibit the use of certain categories of FAs,
- Annex V lists the food colors for which the labelling of foods shall include additional information [28].

Once a food manufacturer has determined the proper classification for their product, they can quickly determine the full list of permitted additives and the concentrations at which they can be used. The union list of FAs (both synthetic and natural additives in Part B section) and their conditions of use appeared in Annexes II (Regulation (EC) No 1129/2011) and III (Regulation (EC) No 1130/2011) under Regulation (EC) No 1333/2008 [28]. The EU allows 363 distinct additives in food, which are specified by number in EU Regulation 1129/2011. These 363 additives are included in Directive 2000/133 and Regulation 510/2013, described in Annex I of Regulation 1333/2008, and have 23 different recognized uses [29].

More recently, steviol glycosides from Stevia were regulated under Regulation (EU) No 2021/1156, amending Annex II to Regulation (EC) No 1333/2008 [30]. FAs Database by EFSA classified approved FAs into four different categories; Group I (additives), Group II (food colors authorized at *quantum statis*), Group III (food colors with combined maximum limit), and Group IV (Polyols), along with their condition of use [31]. EFSA [32] defined sweeteners, colorants, preservatives, and antioxidants based on their functional classifications in Annex I.

When applying for the authorization of a new food additive, the petitioners present their dossier to the European Commission, which must comprise a technical dossier with administrative and risk assessment data, as well as a cover letter. The Commission subsequently sends the dossier to the European Food Safety Authority (EFSA), which will decide on the risk assessment's acceptability within 30 days. If appropriate, the EC will do a validation phase, which will be followed by an EFSA risk assessment that will take no more than 9 months [26]. In this regard, scientific safety data, ADI value, permissible concentrations, and intended uses of FAs need to be well-defined before EU approval. Following the approval, the EC requests that the EFSA issue an opinion on the safety of substances for their intended uses. *In vitro* and *in vivo* animal research, human testing, and epidemiological studies can all be used to assess the safety data. Finally, it can be published in the European Union's Official Journal, making it available to all EU member states. The FDA follows a similar system when it comes to approving new FAs, albeit they don't specify the legal timelines for each procedure [33].

Since 2008, the European Parliament and the Council have set standards for FAs with regulation (EC) No 1333/2008 to ensure food safety, health, and trade throughout Europe, which harmonizes FAs in European countries. In this sense, approved FAs can be revised based on new scientific and technological data. However, NFAs are regulated alongside synthetic ones because there is no other separate legislation for NFAs globally. Unfortunately, sometimes it is unclear how they are made or where their source (synthetic or natural) came from [34].

The use of the color additive is restricted in the EU to specific food groups. Colorants, for example, are expressly prohibited from being added to unprocessed foods, organic-labeled foods, baby food, several EU traditional foods, and unflavored milk, bread, and butter. Colorants of mostly natural origin, listed in the regulation as group II additives, are permitted in certain food categories as *quantum satis*. Color additives used in food products in the EU must be declared by giving the full name and/or E number in the ingredient list. It is illegal to write 'natural color or natural colourant' on labels, neither in the EU nor the USA. Regulations do not distinguish between artificial and natural colorants [35]. According to the European Parliament's Regulation (EC) No 1129/2011, there are 40 colorants approved for use in food in the EU. Anthocyanins (E163), betanin (E162), and carotenoids (E160), including -carotene (E160a), lycopene (E160d), lutein (E161b), canthaxanthin (E161g), chlorophyll and chlorophyllin (E140 and E141), and curcumin (E100) are all regulated colorants from natural sources (according to Regulation (EC) No 1129/2011) [36].

In the last decade, one of the most notable legislative reforms has been the use of biotechnology-derived chemicals with their conditions of use in various food categories. With more additives available today than ever before, there is a growing trend toward regulating the level of a number of low ADI additives [37]. In this regard, NFAs are shown in Table 13.1, based on EU No 1129/2011, (EU) 2021/1156 and (EU) No 0738/2013, amending only Annex II and (EU) No 2020/0771, (EU) No 0232/2012 and (EU) No 2015/1832 amending both Annexes II and III to Regulation (EC) No 1333/2008 [28, 30, 38–42].

13.3 Regulation of Natural Food Additives in the United States

The government of the United States has a lengthy history of intervening to safeguard the food supply. The Food and Drug Administration (FDA) is the federal government's oldest and most comprehensive consumer protection agency [43].

Table 13.3 Natural antimicrobial agents are approved for use in foods for human consumption according to the US FDA regulation

Natural antimicrobial agent[a]	Added to/ Applied on	Origin
Bacteriophage preparation	Meat and poultry	Virus
Lactic acid	All foods, except in infant foods and infant formulas	Bacteria
Natamycin (pimaricin)	Cheese	Bacteria
Nisin	Cheese spreads and pasteurized process cheese spreads with or without fruits, vegetables, or meats	Bacteria
Propionic acid	All foods	Bacteria

[a]All listed substances are GRAS

Driven by concerns about the safety of FAs, the Delaney Committee began an examination into the safety of additives in the US Congress in 1950, which provided the groundwork for the most significant revisions to the US Food, Drug, and Cosmetic (FD&C) Act: The FAs Amendment, which was enacted in 1958, and the Color Additive Amendment, enacted in 1960. They introduced the concept of "GRAS," as "a food substance generally recognized by qualified experts as safe," publishing the first list of GRAS substances containing nearly 200 compounds [44]. Since then, FDA has evaluated and monitored the safety of FAs, although concerns are raised regarding the adequacy of a 60-years old regulation considering the current scientific knowledge in the field [45].

In the face of the increasing demand for natural foods and ingredients, another problem with the current US regulation is the lack of clear definitions and classification between NFAs and SFAs. The Code of Federal Regulation (CFR) only explains the term 'natural' in the section' Food Labeling' (21 CFR 101.22), not in the sections related to FAs and color additives. The abovementioned part of the CFR defines 'natural flavor' or 'natural flavoring' as

> "(…) *the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products, or fermentation products thereof, whose significant function in food is flavoring rather than nutritional. Natural flavors include the natural essence or extractives obtained from plants listed in §§ 182.10, 182.20, 182.40, and 182.50 and part 184 of this chapter and the substances listed in § 172.510 of this chapter*." (21 CFR 101.22(a)(3)).

The FDA has a longstanding policy for not formally defining 'natural.' According to the agency, they have received citizen petitions in the last years requesting the definition of the term and the prohibition of its use in food labeling [46]. Indeed, there are many questions to be addressed before reaching the proper definition of 'natural' when it comes to food, as a claim in food labeling. However, if the agency has already defined 'natural flavors,' why not provide an appropriate definition for 'natural' in the context of the other categories of FAs?

On the other hand, the use of the term 'artificial' can also mislead consumers to wrong interpretations: FDA defines 'artificial color' or 'artificial coloring' as *"any color additive"*(21 CFR 101.22), then includes 'color additives'as *"any material (…) that is a dye, pigment, or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived, with or without intermediate or final change of identity, from a vegetable, animal, mineral, or other source and that, when added or applied to a food, drug, or cosmetic or to the human body or any part thereof, is capable (alone or through reaction with another substance) of imparting a color thereto."* (21 CFR 70.3).

Based on this food labeling regulation, a simple fruit juice, with no additives other than an extract of the same fruit, used as a color enhancer or for color standardization, will have the term 'artificial color' on its label, even if the color additive is from a natural source. In this case, the colorant is natural yet artificially added to the product to change its appearance. Possibly, inattentive or uninformed consumers

do not easily understand this label information and negatively perceive the term 'artificial' with a bad connotation, culminating in product rejection. Thus, a more precise conceptualization of 'artificial' would also greatly benefit food consumers.

Before we move forward to list and discuss some of the NFAs approved by the FDA, we must mention the GRAS regulation (21 CFR 182-186). According to the Final Rule on Substances GRAS [47], the items in this category are not subject to premarket approval. To gain this status, the substance (natural or synthetic) must have general recognition of safety based on scientific procedures or through experience based on common use in foods. If the "common knowledge" argument is used, the notifier must present a substantial history of consumption for food use. However, no GRAS status is conferred if the substance does not satisfy the safety standards for a food additive. And even proven safe and classified as GRAS, not all known NFAs will necessarily be found in the GRAS list. FDA affirms that *"A food ingredient of natural biological origin that has been widely consumed for its nutrient properties (…) without known detrimental effects, which is subject only to conventional processing (…) and for which no known safety hazard exists, will ordinarily be regarded as GRAS without specific inclusion in part 182, part 184 or part 186 of this chapter."* (21 CFR 170.30) [48].

Any person may prepare a petition to voluntarily notify the FDA of a conclusion that a substance is GRAS under the conditions of its intended use. The agency will analyze if the mandatory information and data provided fulfill safety criteria. The list of filed GRAS notices and the FDA letters of responses are made readily accessible to the public in an open database [49]. In the inventory of GRAS notices, the documents provide information on the intended use for each notified substance. However, the purpose of the use is not always available in the GRAS regulatory lists (21 CFR 182, 184, and 186). Natural extracts, spices, and essential oils, for example, are not identified by function. A classification based on specific uses (anticaking, preservatives, sequestrants, stabilizers, nutrients, emulsifiers, and multipurpose food substances) is available only for isolated compounds in part 182 of the document. Unless expressly stated, no use limitation is imposed for GRAS substances in food as long as the producer applies the current good manufacturing practices (GMP).

13.3.1 Natural Colorants

There is an increasing demand for natural colorants in the food industry [50]. Several different pigments can be found in natural sources such as fruits, vegetables, animals, minerals, algae, and microorganisms, which could potentially be used as food colorants if proven safe for consumption.

In the United States, some well-known natural color additives are present in the 'List of Color Additives Exempt from Certification' of the FDA regulation. However, since there is no legal definition for the term 'natural color additive'or 'natural colorant,' the document usually leads consumers and the food industry to the misconception that all additives present in the list are natural, which is not true. The list

includes natural ingredients deliberately used as a food colorant and usually presented in the form of extracts. When added (as a whole or in parts) to foods and contribute to their final color, other naturally colored ingredients (such as berries, chocolate, green or red peppers, and carrots) are not regarded as color additives. They are simply considered food ingredients [51].

NFAs should be subjected to the same strict safety standards as synthetic substances regardless of their source. According to Simon and coworkers, however, noncertified colors must only comply with identity and purity specifications (and use limitations), not being subject to quality and safety examination for the protection of public health before being cleared to be marketed [52].

As a result of the efforts and crescent interest of the food industry in searching novel natural sources of pigments for food application, some natural colorants were recently approved by the FDA, such as spirulina extract, soy leghemoglobin, and butterfly pea flower extract. Table 13.2 highlights natural colorants approved for use in foods in the US. For each of the listed colorants, the regulation has a "uses and restrictions" section indicating the type of food product on which they can be safely applied in amounts consistent with good manufacturing practices.

Although listed as safe for use as a color additive (according to specific limits of addition) and exempt from certification, the natural colorant astaxanthin is not approved for use in human food by FDA. Some natural microbial pigments successfully applied in food products, such as prodigiosin and violacein [53, 54], have not been added to the US regulatory lists of color additives yet. The only direct mention of a color additive derived from microorganisms is riboflavin biosynthesized by *Eremothecium ashbyi*. However, this citation occurs in the CFR section of GRAS substances (21 CFR, §184.1695), not in the color additives section.

Despite the increasing popularity of natural colorants worldwide [55], their safety assessment and quality control can only be guaranteed with a solid harmonized international regulatory framework. Most natural colorants are extracted from complex raw matrices; thus, the use of uniform definitions and standardized criteria for quality control, purity, and safety of these additives would benefit the color additives market and avoid intentional adulteration [52]. This could also encourage research and improve the proposition of novel food-grade natural colorants as well as facilitate their evaluation by the regulatory agencies.

Biotechnological approaches, such as genetically engineered microbial cell factories, have helped the food industry achieve sustainable large-scale production of several natural compounds used as additives [56]. But here is raised another important question related to the construction of consistent regulatory definitions: Can we consider color additives produced by heterologous biosynthesis as natural colorants? This discussion must be resolved in the near future since growing efforts to scale-up production have been using emergent cost-effective and eco-friendly strategies, such as synthetic biology and metabolic engineering [54, 56–58]. An example is soy leghemoglobin, approved and labeled as a color additive in 2019, produced by controlled fermentation by a non-pathogenic and non-toxicogenic genetically modified yeast strain (*Pichia pastoris*). Soy leghemoglobin is not described as a "natural colorant" but is exempt from certification.

Table 13.4 List of food additives from natural origin allowed by Japanese authorities

Name	Main active compound
Annatto extract	norbixin and bixin
Beet red	betanin and isobetanin
Cacao color	polymers of anthocyanins
Carthamus red	carthamin
Clove extract	eugenol
Cochineal extract	carminic acid
Coffee bean extract	chlorogenic acid and polyphenols
Dunaliella carotene	β-carotene
Enzymatically decomposed apple extract	catechins and chlorogenic acid
Grape skin-derived substance	polyphenols
Grape fruit seed extract	fatty acids and flavonoids
Japanese persimmon color	flavonoids
Oregano extract	carvacrol and thymol
Spirulina color	phycocyanin
Tea extract	catechins
Tomato color	lycopene
Turmeric oleoresin	curcumin

Although listed as safe for use as a color additive (according to specific limits of addition)

As previously mentioned, any color additive, either natural or synthetic, must have its safety tested and proved. These substances can only be used upon listing by FDA in the CFR. The Office of Food Additive Safety (OFAS) receives and evaluates the formal petitions, which include the proposition of use and all data from experiments, deciding on the adequacy of the additive in terms of safety and suitability for the intended use [61]. Despite the fact that some authors are not optimistic regarding the search for new natural colors [62], there are currently three petitions under review proposing novel natural colorants: jagua (genipin-glycine) blue, gardenia blue powder, and blue galdieria (*Galdieria sulphuraria*) extract.

13.3.2 Natural Antioxidants

FDA describes 'antioxidants' as *"substances used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation"*(21 CFR 170.3) [63]. Only two natural compounds – gum guaiac and nordihydroguaiaretic acid – are directly mentioned as FDA-approved to be used as 'antioxidant' additives. These NFAs can be isolated from plants and they are used in food packaging [64]. Nevertheless, they are allowed to be added to food packaging and migrate to the food matrix within a specific limit.

Under the label of 'chemical preservatives' in part 184 of GRAS substances, we can find ascorbic acid, erythorbic acid, sorbic acid, and tocopherols — naturally occurring antioxidant substances that are commonly produced by chemical synthesis. Manufacturers prefer synthetic counterparts over natural antioxidants due to their high stability, low cost, and high availability [65].

When using the term 'antioxidant' in the mechanism of search of GRAS notice inventory, the following natural products were found: grape seed extract and grape pomace extract, isoquercitrin, phytic acid, red grape pomace extract, pecan shell fiber, palmitoylated green tea catechins, hesperidin, hydrolyzed aqueous olive pulp extract.

13.3.3 Natural Antimicrobials

Antimicrobial agents are defined by US FDA as *"substances used to preserve food by preventing the growth of microorganisms and subsequent spoilage, including fungistats, mold and rope inhibitors, and the effects listed by the National Academy of Sciences/National Research Council under 'preservatives'"*(21 CFR 170.3) [63]. As previously discussed, there is no distinction, in US regulation, between natural and synthetic antimicrobial additives (or any other food additive, except for flavorings). A list of natural antimicrobial agents approved by the FDA for use in food is presented in Table 13.3.

'*Listeria*-specific bacteriophage preparation' is listed by FDA among FAs permitted for direct addition to food for human consumption. It is approved as an antimicrobial against the pathogenic bacteria *Listeria monocytogenes* by direct spray application to the surface of ready-to-eat meat and poultry products (21 CFR 172.785) [66]. Bacteriophages are ubiquitous viruses that only grow in bacteria, meaning that they cause no harm to humans and other animals. They are valuable candidates for detecting and controlling contamination because of their innate specificity to infect and kill their target bacteria [67].

Although known to take part in the food fermentation process, lactic acid bacteria (LAB) can also be used for biopreservation [33]. They indirectly impair the growth of spoilage and pathogenic bacteria by reducing food pH or affecting them by the production of bacteriocins, a group of antimicrobial peptides [68]. FDA classified several LAB as GRAS, which means that live bacteriocin-producing LAB can potentially be used as antimicrobial additives. A GRAS notice dated 2005 proposes using a LAB mixture in fresh meat and carcasses of beef and poultry at use levels between 10^6 to 10^8 colony-forming units of lactobacilli per gram of product. The intended use was "to control the growth of pathogenic bacteria."

Several substances from natural sources with antimicrobial activity are listed in the GRAS notice inventory: lactoferrin, lysozyme, hops beta acids, polylysine, citrus fruit extract, glycolipids from *Dacryopinax spathularia*, and many preparations of bacteriophages. Other antimicrobial agents of different sources, such as essential oils, extracts, spices, and herbs, meet the eligibility criteria for classification as

GRAS and are present in lists provided by the regulation (21 CFR 182). The GRAS regulation does not include a separate list of isolate compounds classified as 'antimicrobial agents.'

13.3.4 Natural Sweeteners

The US food regulatory document for FAs subdivides sweeteners into nutritive and non-nutritive. Nutritive sweeteners are considered as *"substances having greater than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity"* and non-nutritive sweeteners are described as *"substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity"* [63]. No special sections are dedicated to describing and listing the substances in these two groups. As discussed in this chapter, no distinction regarding natural or synthetic origin is presented in the US regulation related to this group of additives.

Besides honey, a natural product, other well-known and commonly used nutritive carbohydrate sweeteners are cane sugar, beetroot sugar, and corn syrup. Nonetheless, for obvious reasons related to the ultra-processing procedures their plant raw material undergoes, the 'naturalness'of these three ingredients is highly questionable (although defined as GRAS by FDA). Cane sirup, maple sirup, and sorghum sirup, defined by Castro-Muñoz and coworkers as 'natural sweeteners' [69], are listed in the 'Sweeteners and Table Sirups' section (Part 168).

GRAS notices for sweeteners from natural sources (nutritive and non-nutritive) are registered in the GRAS database. They include agave extract (*Agave tequilana*), steviol glycosides obtained from the stevia plant (*Stevia rebaudiana* Bertoni), extracts of Swingle fruit (*Siraitia grosvenorii*), and thaumatin — a protein originally expressed by the plant *Thaumatococcus daniellii*, but industrially produced by genetic engineering processes. The sugars erythritol, D-tagatose, and trehalose also appear in the GRAS notice inventory. As explained in the database documents, these are naturally occurring sugars usually produced by fermentation or enzymatic synthesis.

13.4 Regulation of Natural Food Additives Based on ISO TS 19657:2017

The issue of the naturalness of food ingredients often leads to inconsistencies. In this context, a technical specification was published by the International Organization for Standardization (ISO) in December 2017, which includes the necessary definitions and technical requirements for food ingredients to be described "natural." Despite consumers' interest in "natural foods," no internationally accepted

regulatory standard has been announced for the use of the term "natural" (with the exception of natural mineral water and natural flavors) until the ISO/TS 19657:2017 technical specification is published [70]. The ISO/TS 19657:2017 technical specification aimed to define the natural ingredient. Thus, this specification has provided technical criteria to which businesses can apply to use the term "natural."

ISO/TS 19657:2017 technical specification [15]:

It has defined technical requirements for food ingredients that can be described as natural. However, this document does not contain all the factors that influence the naturalness of food. For this reason, a more comprehensive specification should be prepared to meet the needs of consumers.

- It contains basic guidelines for the food and beverage industry. Thus, this specification is intended to help the food and beverage industries communicate in the same language.
- While it can help with business-to-business (B2B) communication, it cannot help with consumer-to-product communication.
- Natural mineral waters, bottled drinking water, and flavorings are outside the scope of this technical specification.

According to the ISO/TS 19657:2017 technical specification, it must meet the following criteria for a food ingredient to be described as natural [15].

(a) It is stated that for a food ingredient to be considered natural, it must be obtained from "plant, algae, fungus, animal, microorganism, mineral deposits or seawater." It has also been stated that fossil fuels will not be used as a source.
(b) Physical, enzymatic, and microbiological processing methods are allowed for food ingredients to be considered natural. However, the specification additionally states that "enzymatic or microbiological processes should not be used to produce substances not found in nature." It has also been added that pH adjustments are allowed in these processes.
(c) It is stated that if the above-mentioned methods cannot be applied in the processing of foods, other processes can be used "to meet food safety or regulatory requirements." In addition, it was stated that food components should not be changed by processes.

In addition to the above criteria, it is stated that the removal and addition of water during processing or the removal of any component from the food ingredient "does not affect the naturalness of the food content."

13.5 Regulation of Natural Food Additives in Other Countries

Although the definition of a food additive varies from country to country, it is generally defined as a substance added to achieve a technological function in the final product. Apart from the USA and the EU, there are other regulatory systems for

other countries that regulate FAs and examine their safety. Before these regulatory systems publish the list of permitted FAs, they research their safety and determine the criteria for conditions of use. If the use of a food additive is not allowed, the applicant who wants to use this food additive must apply in accordance with the regulatory rules for the approval of its use in the relevant country [71]. Although there are important developments in terms of food additives, the lack of uniformity in the regulations of additives between countries causes contradictions.

- Ministry of Health, Labor and Welfare of Japan (MHLW), Food Standards Australia New Zealand (FSANZ), and the Food Safety and Standards Authority of India (FSSAI) are examples of organizations dealing with food regulations in other countries [72].

13.5.1 Japan

The Food Sanitation Act (FSA), passed in 1947 by the MHLW, is the first comprehensive Act for food safety. All FAs have been regulated by this act since 1947 [73]. The list of FAs of natural origin in Japan was first published by the MHLW on April 16, 1996, and this list has been updated several times. A total of 357 NFAs are listed as of February 26, 2020 [74]. Some of this list is given in Table 13.4 as an example [65].

13.5.2 Australia New Zealand

FSANZ is in charge of defining and managing food standards in Australia and New Zealand. FSANZ is governed by a Board that works for the Australian and New Zealand Food Regulation Ministerial Council. The standards developed by FSANZ are defined by the Australian New Zealand Food Standards Act [75]. There is no specific regulation regarding NFAs, but they are regulated by existing legal and regulatory frameworks.

13.5.3 India

In India, FAs are regulated by the FSSAI. In 2006, the FSSAI developed six key regulations and came into force on 5 August 2011. It is updated when necessary [76]. FSSAI published the "Food Safety and Standards (Food Products Standards and Food Additives) Regulation" as the regulatory standard for FAs in 2011. In this regulation, natural colorants and natural flavorings are mentioned in relation to NFAs. There is a list of natural colorants. This list includes chlorophyll, caramel,

curcumin or turmeric, carotene and carotenoid, canthaxanthin, riboflavin, annatto, saffron. However, there is no list of natural flavorings in this regulation. In addition, additives such as grape skin extract, paprika extract, natural tocopherol are also on the list. However, no special classification has been made for these, and they are among other additives [77].

13.5.4 Mercosur

Mercosur was founded in 1991 by Argentina, Brazil, Paraguay, and Uruguay. Venezuela joined as a member in 2012, but its membership was suspended in 2016 [78]. Mercosur standards are influenced by the EU, Codex Alimentarius, and FDA [79]. In some cases, they have adopted these standards; in others, they have introduced the necessary changes. Harmonization of national food standards has been undertaken by Argentina, Brazil, Paraguay, and Uruguay [78]. These countries are generally improving their food regulation systems and replacing them with official Mercosur standards [80]. Additives were assigned to their categories after long and tedious discussions. This slow progress has been slow due to different raw materials available in member countries, different processing and packaging equipment, storage and distribution conditions, consumer dietary habits, and consumer expectations. The first harmonized list was approved in 1993 [81], and the last revision was made in 2006 [82]. Several natural substances are approved for use as FAs and present in the harmonized list. Nevertheless, there is no classification based on their sources nor the use of the 'natural' term.

The Brazilian "Agência Nacional de Vigilância Sanitária" (ANVISA) uses the Mercosur standards as a basis for its internal regulations, but complementary regulations and list updates are frequently released. The last published updates included the permission to use tocopherols [83], steviol glycosides, and microbial β-carotene in some classes of foods [84].

13.6 Future Perspectives for New Products

Consumers are eager to purchase clean-labelled, safe, healthy, and no-synthetic-added foods. To meet this desire, food manufacturers can reformulate their products with NFAs to have healthy, safe, and sustainable food products [23, 85, 86]. NFAs are also popular for their added benefits, such as bioactivity and nutraceutical characteristics [10]. Based on the recent studies, essential oils [87, 88] and natural antimicrobials [33] in films and coatings, as well as bio-additives and -colorants [56], natural sweeteners [69], natural antioxidants [34], and their combinations, are promising alternatives as natural FAs that can be used in and on foods once the European Commission (EC) registers them. FAs can be introduced directly at controlled levels (e.g., the low-calorie sweetener aspartame in yogurt, beverages,

chewing gum, puddings [89]) or indirectly (e.g., trace amounts of coating or paper material transferred into food via packaging, storage, and handling) to serve a technical function [90].

The valorization of agricultural by-products can be used as natural antioxidants [91]. For example, pomegranate peel extract was proposed to be used as a natural food additive with its strong antioxidant and antimicrobial traits without affecting the sensory properties of foods. Still, toxicity and safety data are required to establish allowable limits [92]. Additionally, kiwi [93] and citrus peel waste [94] can be utilized as natural FAs. Green (without using harsh chemicals) extraction of natural antioxidants is important. For instance, phenolic compounds from grape pomace were obtained with ultrasound-assisted extraction and water as a safe solvent [95]. Similarly, pomace extract exhibited strong antibacterial effects on *Listeria monocytogenes* and *Staphylococcus aureus* [96]. With their possible toxicity and carcinogenicity, synthetic antioxidants (e.g., butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ)) might be substituted with natural alternatives. In this context, rosemary extract (E392) was approved in meat/seafood products and oil/fat [97].

Natural antimicrobials can be used in foods to combat foodborne pathogens or spoilage bacteria, implying a clean-label and healthy food approach [98]. Some natural antimicrobials from plants (spices and herbs, essential oils, antioxidants), animals (chitosan, lysozyme, pleurocidin, curvacin A, spheniscin, free fatty acids, magainin-antimicrobial peptide, lactoperoxidase, and lactoferrin), and microorganisms (bacteriocins, nisin, pediocin, reuterin, bacteriophages, yeasts) can be utilized in foods instead of synthetic/chemical counterparts [33]. For example, natamycin (E 235) is a GRAS antimicrobial used in ready-to-drink tea beverages; fruit-flavored energy, sport, isotonic drinks; fruit-flavored drinks [99]. The EU authorizes a maximum of 1 mg/dm^2 of natamycin as an additive on the surface of semi-hard and semi-soft cheese rinds [28]. Natural antimicrobials can be added to foods directly (as an ingredient) or indirectly (via packaging or coating materials) through the active packaging technique [98]. Similarly, nisin (E 234) from *Lactococcus lactis* is a GRAS bio-preservative against harmful gram-positive microorganisms, and its combination with active packaging and non-thermal (such as pulsed electric field) applications can broaden its antimicrobial activity on gram-negatives [100, 101]. Likewise, the combined application of reuterin, lactoperoxidase, and lactoferrin inactivated food-borne pathogens (with 5 log *Salmonella* Enteritidis and *E. coli* O157:H7, and 0.8 log CFU/g reduction for *Listeria monocytogenes*) in cooked ham under high hydrostatic pressure (450 MPa for 5 min) [102]. Furthermore, lysozyme (E 1105) is a GRAS [103] antimicrobial enzyme that can lyse the cell wall of Gram-positive bacteria's peptidoglycan structure due to their high peptidoglycan content [104, 105]. Lactoperoxidase, another antimicrobial enzyme with bactericidal and bacteriostatic activity against various microorganisms, was widely used in the dairy industry [106]. Lactoferrin is an antimicrobial protein with an iron-binding property used to combat pathogenic microorganisms (including *L. monocytogenes, Salmonella spp, Escherichia coli, Bacillus stearothermophilus, Shigella dysenteriae,* and *Bacillus subtilis*) found in meat, dairy, beverages, bakery, and seafood

[107]. Natural antimicrobials can be combined to make use of 'hurdle technology'; for example, nisin (12.5 mg/kg), natamycin (200 mg/kg), green tea extract (0.2%), and citric acid (pH 3.5) have extended the shelf life of fruit and vegetable smoothies by 14 days while also controlling *Listeria monocytogenes* contamination (6 log CFU/mL) [108]. Importantly, with antibiotic resistance on the rise, lactoferrin-derived peptides and plant extracts (terpenoid, alkaloid, and phenolic chemicals) are promising candidates for novel natural antimicrobial agents [109].

Natural sweeteners can be sourced from various compounds, such as sugars, sugar alcohols (polyols; mannitol, sorbitol, erythritol, and xylitol), amino acids or proteins, terpenoid glycosides, and some polyphenols [85, 110]. Natural sweeteners (e.g., glucose, fructose, sucrose, and steviol glycosides) are gaining popularity as healthier alternatives to their artificial (e.g., aspartame, saccharin, sucralose, acesulfame-potassium, cyclamate, alitame, neotame, and dulcin) semi-artificial (e.g., neohesperidine dihydrochalcone) equivalents without sacrificing flavor or functional characteristics. Natural sweeteners such as honey, sugar alcohols (e.g., erythritol, mannitol, and xylitol), stevia (steviol glycosides), molasses, agave nectar, coconut sugar, date syrup, maple syrup, and sorghum syrup; are claimed to have a higher nutritional value, resulting in a lower glycemic index, which also offers added health benefits due to their high vitamin and mineral content [69, 85]. In addition, monk fruit, yacon syrup, carob syrup, and palm sugar were also considered natural sweeteners in yoghurt, skim milk, and chocolate products. Notably, natural sweeteners'utilization is highly dependent on their organoleptic properties, structure, and texture [69]. In addition to intrinsic (nutritive value, sweetening power) and origin (synthetic, semisynthetic, and natural) qualities, thermal stability and hydrophilic nature are essential factors for sweetness potency [85]. In this context, sucrose is the most commonly used sweetener (sweetness potency = 1) [85], although it can lead to dental problems, diabetes, cancer, and obesity [111].

Sweeteners are categorized into two categories depending on their relative sweetness: bulk (e.g., Erythritol) and intense (e.g., Aspartame). Bulk sweeteners have equal or lower sweetening potencies than sucrose, whereas intense sweeteners have a higher potency [85]. Besides, natural sweeteners such as erythritol (E 968), tagatose (no E number), steviol glycosides (E 960), and thaumatin (E 957) can be produced through eco-friendly approaches. Moreover, sugar alcohols such as erythritol, mannitol, and xylitol are also classified as natural sweeteners and are widely used in soft drinks, gum, candy, and baked products. Furthermore, sugar alcohols act as prebiotics, improving beneficial microbes in gut microbiota, and are generally considered safe. Their consumption within the acceptable daily intake (ADI) levels does not pose a health risk. On the other hand, sugar alcohols can be partially metabolized compared to sucrose and glucose, and their excessive consumption may result in laxative symptoms [85]. In this context, erythritol was found in various fruits (for example, watermelons, pears, and grapes), classified as GRAS [112]. Erythritol accounts for approximately 65% of the sweetness of sucrose, which can also be produced through the fermentation of fungi or lactic acid bacteria, followed by purification. Similarly, trehalose (naturally occurring in plants, fungi, insects, and yeasts and composed of two glucose units with α-1,1-glycosidic bond) and

tagatose (fructose isomer naturally occurring in some fruit and dairy products) are sucrose substitutes [34, 85]. Likewise, tagatose (with 92% of sweetness potency) can be converted from D-galactose to D-tagatose via enzymatic reaction, which was approved as GRAS [113] prebiotic monosaccharide to be used as a flavor enhancer in cereals, diet soft drinks, chocolate, soft/hard confectionery, ice cream, yoghurt, chewing gum, frostings and dietary supplements [114].

However, artificial sweeteners have fewer or zero calories than sugar and can adversely alter the microbiome [115], resulting in reduced glucose tolerance. Although there are FDA- (aspartame, neotame, saccharin, acesulfame-K, sucralose, and advantame) and EFSA- (acesulfame-K (E 950), aspartame (E 951), cyclamate (E 952), saccharin (E 954), sucralose (E 955), thaumatin (E 957), neohesperidine DC (E 959), steviol glycosides (E 960), neotame (E 961), aspartame-acesulfame salt (E 962), advantame (E 969)) approved sweeteners, several safety controversies on artificial sweeteners' safety [116] have made their use debatable and optional. Aspartame, the first FDA-approved sweetener, was found to cause oxidative stress in blood cells, leading to hepatotoxicity and kidney failure. In addition, aspartame is not recommended for phenylketonuria patients due to its phenylalanine content [117]. However, several studies have shown that aspartame has no negative effects on blood pressure and is a suitable replacement for type 2 diabetes [118]. A similar controversial situation is available for sucralose, saccharin, and acesulfame-K. In this sense, sucralose is roughly 600 times sweeter than sugar and is assumed to be indigestible by humans; nevertheless, investigations have revealed that it may be digested. Increasing glucose and insulin levels can lead to diabetes [119, 120]. Similarly, saccharin was found to cause bladder cancer and was banned in Canada. The FDA and EFSA approved it after several studies refuted the claims about its carcinogenic risk. A similar tendency was observed for acesulfame-K, which cannot be digested by humans and is 120 times sweeter than sugar, but was thought to be safe at low concentrations [69]. Furthermore, saccharin, acesulfame-K, and aspartame can damage DNA in human peripheral lymphocytes [121], and non-caloric artificial sweeteners can adversely affect the brain, restricting its usage for lengthy periods [122]. The usage of new/potential natural sweeteners instead of artificial sweeteners is becoming increasingly popular.

13.7 Conclusions

The lack of a universal definition of 'natural' generates disagreements between regulatory agencies worldwide regarding food additives, potentially misleading food-chain suppliers, manufacturers, and consumers. Intense technological advances in the food industry and the globalized food trade urge the search for a consensus in the use of the term 'natural,' not only for labeling purposes but also for food ingredients production and marketing, including additives. This movement will be essential to the international food market and to avoiding litigation disputes about "natural" claims in food labeling.

Independently of the need to extinguish ambiguity around the term ‘natural’ in the food industry, consumers education is a pivotal point if we want to help people with healthy eating habits. One of the reasons for the reluctance of regulatory agencies to adopt a wide use of the term ‘natural’ is probably the consumer’s misconception that what is natural is always safer, purer, healthier, and, consequently, of superior quality. Government, academia, health workers, food industry, and merchandising companies all have a role in providing science-based, reliable, and precise information to the population, demystifying the term ‘natural,’ using language accessible to all types of audiences.

With no means to verify the natural status of food and its ingredients, consumers rely only on the government regulations to protect them from food industries and retailers seeking economic advantages under the misuse of ‘natural’ claims. Therefore, it is crucial that regulatory documents include clear and harmonized definitions for natural additives, avoiding multiple interpretations. Regular revisions are also imperative, considering the constant increment of novel processing technologies, products, and ingredients, thanks to food science and technology advances.

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References

1. Food additives. World Health Organization. p. 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/food-additives.
2. World Health Organization,Food additives. 2018.
3. TheJointFAO/WHOExpertCommitteeonFoodAdditives(JECFA).2022.Availablefrom:https://www.who.int/groups/joint-fao-who-expert-committee-on-food-additives-(jecfa)/about.
4. U.S. Food and Drug Administration. 2022. Available from: vhttps://www.fda.gov/.
5. European Food Safety and Authority (EFSA). 2022. Available from: https://www.efsa.europa.eu/en.
6. Japan Ministry of Health, Labour and Welfare. 2022. Available from: https://www.mhlw.go.jp/english/.
7. Food Safety and Standards Authortiy of India. 2022. Available from: https://www.fssai.gov.in/.
8. Mercosur (Mercado Común del Sur). 2022. Available from: https://www.mercosur.int/en/.
9. International Food Addditives Council, Food safety and regulation, Other Nations. 2022.
10. Carocho M, Barreiro MF, Morales P, Ferreira ICFR. Adding molecules to food, pros and cons: a review on synthetic and natural food additives. Compreh Rev Food Sci Food Safety. Blackwell Publishing Inc. 2014;13:377–99.
11. Rozin P. The meaning of “natural”: process more important than content. Psychol Sci. 2005;16(8):652–8. Available from: https://journals.sagepub.com/doi/abs/10.1111/j.1467-9280.2005.01589.x.
12. Lang M, Rodrigues AC. A comparison of organic-certified versus non-certified natural foods: perceptions and motives and their influence on purchase behaviors. Appetite. 2022;168:105698. Available from: https://www.sciencedirect.com/science/article/pii/S019566632100605X

13. Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC. Off J Eur Union. 2008;354:34–50.
14. Food Safety and Inspection Service, Meat and poultry labeling terms. 2013. Available from: https://www.fsis.usda.gov/wps/portal/fsis/top ics/food-safety-education/get-answers/food-safety-fact-sheets/food-labeling/m eat-and-poultry-labeling-terms/meat-and-poultry-labeling-terms/!ut/p/a1/jZ FRb4IwEMc_DY-lx3AG90ZIFmUTZsxm5WUpehSS0pK2jrhPP9wyExed9p.
15. ISO/TC 34 Food products. ISO/TS 19657:2017 - Definitions and technical criteria for food ingredients to be considered as natural. ISO Tech Specif. 2017;2017.
16. Battacchi D, Verkerk R, Pellegrini N, Fogliano V, Steenbekkers B. The state of the art of food ingredients' naturalness evaluation: a review of proposed approaches and their relation with consumer trends. Trends Food Sci Technol. Elsevier Ltd. 2020;106:434–44.
17. European Commission. Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. Off J Eur Union. 2012.
18. Baines D. Defining the term 'natural'in the context of food products. Nat Food Addit Ingredients Flavourings. 2012, 2012:1–22.
19. European Commission. Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. Off J Eur Union. 2008;1–6.
20. Food additive re-evaluations. European Food Safety Authority. 2022. Available from: https://www.efsa.europa.eu/en/topics/topic/food-additive-re-evaluations.
21. EFSA Regulation. European food Safety Authority,Re-evaluation of food additives under Regulation (EC)No 257/2010. 2019.
22. EFSA Regulation. European food Safety Authority,Sweeteners. 2022. Available from: https://www.efsa.europa.eu/en/topics/topic/sweeteners.
23. Battacchi D, Verkerk R, Pellegrini N, Fogliano V, Steenbekkers B. The state of the art of food ingredients' naturalness evaluation: a review of proposed approaches and their relation with consumer trends. Trends Food Sci Technol. 2020;106(July):434–44. Available from: https://doi.org/10.1016/j.tifs.2020.10.013.
24. Román S, Sánchez-Siles LM, Siegrist M. The importance of food naturalness for consumers: results of a systematic review. Trends Food Sci Technol. 2017;67:44–57. Available from: https://www.sciencedirect.com/science/article/pii/S092422441730122X.
25. Debeuckerlaer W. Legislation on food additives, food enzymes and flavourings in the European Union. Curr Opin Food Sci. Elsevier Ltd. 2015;6:49–52.
26. Ferreira ICFR, Carocho M. Food and nutritional analysis | Food additives. In: Encyclopedia of analytical science. 3rd ed. Elsevier Inc; 2019. p. 419–427. Available from: https://doi.org/10.1016/B978-0-12-409547-2.14310-0
27. Lehto S, Buchweitz M, Klimm A, Straßburger R, Bechtold C, Ulberth F. Comparison of food colour regulations in the EU and the US: a review of current provisions. Food Addit Contam - Part A Chem Anal Control Expo Risk Assess. 2017;34(3):335–55. Available from: https://doi.org/10.1080/19440049.2016.1274431
28. European Commission. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Off J Eur Union. 2009;51:16–33. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02008R1333-20210808&qid=1643895326386&from=en#tocId44
29. Saltmarsh M. Recent trends in the use of food additives in the United Kingdom. J Sci Food Agric. 2015;95(4):649–52.
30. Commission Regulation (EU) 2021/1156 of 13 July 2021 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to

Commission Regulation (EU) No 231/2012 as regards steviol glycosides (E 960) and rebaudio. Off J Eur Union. 2021.
31. Food Additives Database. European Commission. 2022. Available from: https://webgate.ec.europa.eu/foods_system/main/?event=substances.search&substances.pagination=1.
32. European Parliament and the Concil of the European Union. Regulation (EC) No 1333/2008 of the European Parliament ans of the Council of 16 December 2998 on food additives. Off J Eur Union. 2008;(L 354):16–33.
33. El-Saber Batiha G, Hussein DE, Algammal AM, George TT, Jeandet P, Al-Snafi AE, et al. Application of natural antimicrobials in food preservation: recent views. Food Control. 2021;126(January):108066.
34. Carocho M, Morales P. Ferreira ICFR. Natural food additives: Quo vadis? Trends Food Sci Technol. Elsevier Ltd. 2015;45:284–95.
35. Lehto S, Buchweitz M, Klimm A, Straßburger R, Bechtold C, Ulberth F. Comparison of food colour regulations in the EU and the US: a review of current provisions. Food Addit Contam - Part A Chem Anal Control Expo Risk Assess. 2017;34(3):335–55.
36. Faustino M, Veiga M, Sousa P, Costa EM, Silva S, Pintado M. Agro-food byproducts as a new source of natural food additives. Molecules. 2019;24(6):1–23.
37. Blekas GA. Food additives: classification, uses and Regulation. 1st ed. Encyclopedia of food and health. Elsevier Ltd; 2015. p. 731–736.
38. EFSA-Food Additives Database. 2022. Available from: https://webgate.ec.europa.eu/foods_system/main/?sector=FAD&auth=SANCAS.
39. Commision Regulation (EU) No 738/2013 of 30 July 2013 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the use of certain additives in seaweed based fish roe analogues. Off J Eur Union. 2013.
40. Commission Regulation (EU). 2020/771 of 11 June 2020 amending Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No 231/2012 as regards the use of Annatto, Bixin, Nor. Off J Eur Union. 2020.
41. Commission Regulation (EU) No 232/2012 of 16 March 2012 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the conditions of use and the use levels for Quinoline Yellow (E 104), Sunset Yellow FCF/Ora. Off J Eur Union. 2012.
42. Commission Regulation (EU) 2015/1832 of 12 October 2015 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the use of Erythritol (E 968) as a flavour enhancer in energy-reduced or with no added sugar. Off J EurUnion. 2015.
43. US FDA. FDA History. 2018. Available from: https://www.fda.gov/about-fda/fda-history.
44. Meadows M. A century of ensuring safe foods and cosmetics FDA Consumer magazine The Centennial Edition. FDA Consum. 2006;40(1):6–13. Available from: www.fda.gov
45. Krishan M, Navarro L, Beck B, Carvajal R, Dourson M. A regulatory relic: after 60 years of research on cancer risk, the Delaney Clause continues to keep us in the past. Toxicol Appl Pharmacol. 2021;433(October):115779. Available from: https://doi.org/10.1016/j.taap.2021.115779
46. Use of the Term Natural on Food Labeling. Food and Drug Administation. 2018.
47. US FDA. Substances generally recognized as safe - final rule. Fed Regist. 2016;81(159) Available from: https://www.federalregister.gov/documents/2016/08/17/2016-19164/substances-generally-recognized-as-safe.
48. US FDA. Eligibility for classification as generally recognized as safe (GRAS), 21 C.F.R. § 170.30. 2016.
49. US FDA. GRAS Notices. 2022. Available from: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=GRASNotices.

50. Landim Neves MI, Silva EK, Meireles MAA. Natural blue food colorants: consumer acceptance, current alternatives, trends, challenges, and future strategies. Trends Food Sci Technol. 2021;112(February):163–73. Available from: https://doi.org/10.1016/j.tifs.2021.03.023%0A
51. US FDA. Color Additives, 21 C.F.R. § 70.3. 2022.
52. Simon JE, Decker EA, Ferruzzi MG, Giusti MM, Mejia CD, Goldschmidt M, et al. Establishing standards on colors from natural sources. J Food Sci. 2017;82(11):2539–53. Available from: https://doi.org/10.1111/1750-3841.13927
53. Rana B, Bhattacharyya M, Patni B, Arya M, Joshi GK. The realm of microbial pigments in the food color market. Front Sustain Food Syst. 2021;5(March 2021):1–14.
54. Sen T, Barrow CJ, Deshmukh SK. Microbial pigments in the food industry—challenges and the way forward. Front Nutr. 2019;6(March):1–14.
55. Martins N, Roriz CL, Morales P, Barros L, Ferreira ICFR. Food colorants: challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. Trends Food Sci Technol. 2016;52:1–15.
56. Sun L, Xin F, Alper HS. Bio-synthesis of food additives and colorants-a growing trend in future food. Biotechnol Adv. 2021;47(January):107694. Available from: https://doi.org/10.1016/j.biotechadv.2020.107694
57. Sankari M, Rao PR, Hemachandran H, Pullela PK, Doss CGP, Tayubi IA, et al. Prospects and progress in the production of valuable carotenoids: insights from metabolic engineering, synthetic biology, and computational approaches. J Biotechnol. 2018;266(July 2017):89–101.
58. Frandsen RJN, Khorsand-Jamal P, Kongstad KT, Nafisi M, Kannangara RM, Staerk D, et al. Heterologous production of the widely used natural food colorant carminic acid in Aspergillus nidulans. Sci Rep. 2018;8(1):1–10.
59. Rebelo BA, Farrona S, Rita Ventura M, Abranches R. Canthaxanthin, a red-hot carotenoid: applications, synthesis, and biosynthetic evolution. Plan Theory. 2020;9(8):1–18.
60. Averianova LA, Balabanova LA, Son OM, Podvolotskaya AB, Tekutyeva LA. Production of Vitamin B2 (Riboflavin) by microorganisms: an overview. Front Bioeng Biotechnol. 2020;8(November):570828.
61. US FDA. Color Additive Petitions. 2019. Available from: https://www.fda.gov/industry/color-additives/color-additive-petitions.
62. Coultate T, Blackburn RS. Food colorants: their past, present and future. Color Technol. 2018;134(3):165–86.
63. US FDA. Food Additives, 21 C.F.R. § 170.3 . 1977. Available from: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-170.
64. US FDA. Prior-sanctioned Food Ingredients, 21 C.F.R. § 181.24. 1977.
65. Gutiérrez-Del-río I, López-Ibáñez S, Magadán-Corpas P, Fernández-Calleja L, Pérez-Valero Á, Tuñón-Granda M, et al. Terpenoids and polyphenols as natural antioxidant agents in food preservation. Vol. 10, Antioxidants. MDPI; 2021.
66. US FDA. Food additives permitted for direct addition to food for human consumption, 21 C.F.R. § 172.785. 2016.
67. Endersen L, Coffey A. The use of bacteriophages for food safety. Curr Opin Food Sci. 2020;36:1–8. Available from: https://doi.org/10.1016/j.cofs.2020.10.006
68. Quinto EJ, Caro I, Villalobos-Delgado LH, Mateo J, De-Mateo-silleras B, Redondo-Del-río MP. Food safety through natural antimicrobials. Vol. 8, Antibiotics. MDPI AG; 2019.
69. Castro-Muñoz R, Correa-Delgado M, Córdova-Almeida R, Lara-Nava D, Chávez-Muñoz M, Velásquez-Chávez VF, et al. Natural sweeteners: sources, extraction and current uses in foods and food industries. Food Chem. 2022;370(January 2021):130991.
70. Schifferstein HNJ, de Boer A, Lemke M. Conveying information through food packaging: a literature review comparing legislation with consumer perception. J Funct Foods. 2021;86(August):104734.
71. Magnuson B, Munro I, Abbot P, Baldwin N, Lopez-Garcia R, Ly K, et al. Review of the regulation and safety assessment of food substances in various countries and jurisdictions. Food Addit Contam - Part A Chem Anal Control Expo Risk Assess. 2013 Jul;30(7):1147–220.

72. İnternational Food Additives Council (ifac). Food Safety & Regulation.
73. MHLW. Food Additives. [cited 2022 Feb 12]. Available from: https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/kenkou_iryou/shokuhin/syokuten/index_00012.html.
74. The Japan Food Chemical Research Faundation. List of existing food additives. The Japan Food chemical Research Faundation; 2020.
75. Federal Register of Legislation. Food Standards Australia New Zealand Act 1991.
76. Mota IGC, Das NRAM, Nascimento SSDC, Maciel BLL, Morais AHDA, Passos TS. Artificial dyes: health risks and the need for revision of international regulations. Food Rev Int. 2021;00(00):1–16.
77. fssai. Food Safety and Standards Regulations. [cited 2022 Feb 21]. Available from: https://fssai.gov.in/cms/food-safety-and-standards-regulations.php.
78. Gallagher J, McKevitt A. Laws and regulations of traditional foods: past, present and future. In: Al-Khusaibi M, Al-Habsi N, Shafiur Rahman M, editors. Traditional food (food engineering series). Cham: Springer; 2019. p. 239–71.
79. Vapnek J, Purnhagen K, Hillel B. Regulatory and legislative framework for novel foods. In: Pathania S, Tiwari BK, editors. Food formulation: novel ingredients and processing techniques. Wiley; 2021.
80. Magnuson B, Munro I, Abbot P, Baldwin N, Lopez-Garcia R, Ly K, et al. Review of the regulation and safety assessment of food substances in various countries and jurisdictions. Food Addit Contam - Part A Chem Anal Control Expo Risk Assess. 2013;30(7):1147–220.
81. De F, Toledo MC. A need for harmonized legislation: perspectives in South America. J Sci Food Agric. 2014;94(10):1958–61.
82. MERCOSUR. MERCOSUR/GMC/RES. Nº 11/06. Reglamento Tecinoco MERCOSUR sobre "Lista General Armonizada de Aditivos Alimentarios y Sus Clases Funcionales." 2006.
83. BRASIL. Agência Nacional de Vogilância Sanitária (ANVISA) - RDC N° 397, de 25 de junho de 2020. Autoriza o uso de aditivos alimentares em diversas categorias de alimentos. Diário Of da União. 2020;124.
84. BRASIL. Agência Nacional de Vigilância Sanitária. RDC N° 588, de 20 de dezembro de 2021. Autoriza o uso de aditivos alimentares e coadjuvantes de tecnologia em diversas categorias de alimentos. Diário Of da União 2021;240.
85. Saraiva A, Carrascosa C, Raheem D, Ramos F, Raposo A. Natural sweeteners: the relevance of food naturalness for consumers, food security aspects, sustainability and health impacts. Int J Environ Res Public Health MDPI AG. 2020;17:1–22.
86. Luo M, Zhou DD, Shang A, Gan RY, Bin LH. Influences of food contaminants and additives on gut microbiota as well as protective effects of dietary bioactive compounds. Trends Food Sci Technol. 2021;113(January):180–92.
87. Sharma S, Barkauskaite S, Jaiswal S, Duffy B, Jaiswal AK. Development of essential oil incorporated active film based on biodegradable blends of poly (Lactide)/poly (butylene Adipate-co-terephthalate) for food packaging application. J Packag Technol Res. 2020;4(3):235–45. Available from: https://doi.org/10.1007/s41783-020-00099-5
88. Pateiro M, Barba FJ, Domínguez R, Sant'Ana AS, Mousavi Khaneghah A, Gavahian M, et al. Essential oils as natural additives to prevent oxidation reactions in meat and meat products: a review. Food Res Int. 2018;113:156–66.
89. U.S. Department of Health & Human Services (HHS) Food and Drug Administration (FDA). What is a food additive? 2022.
90. Gilsenan MB. Additives in dairy foods | Safety. In: Second E, editor. Fuquay JWBT-E of DS. San Diego: Academic; 2011. p. 55–60. Available from: https://www.sciencedirect.com/science/article/pii/B9780123744074000054.
91. Faustino M, Veiga M, Sousa P, Costa EM, Silva S, Pintado M. Agro-food byproducts as a new source of natural food additives. Vol. 24, Molecules. MDPI AG; 2019.
92. Kaderides K, Kyriakoudi A, Mourtzinos I, Goula AM. Potential of pomegranate peel extract as a natural additive in foods. Trends Food Sci Technol. Elsevier Ltd. 2021;115:380–90.

93. Chamorro F, Carpena M, Fraga-Corral M, Echave J, Riaz Rajoka MS, Barba FJ, et al. Valorization of kiwi agricultural waste and industry by-products by recovering bioactive compounds and applications as food additives: a circular economy model. Food Chem. 2022;370(October 2021):131315.
94. Matsuo Y, Akita K, Taguchi H, Fujii S, Yoshie-Stark Y, Araki T. Utilization and evaluation of Citrus natsudaidai peel waste as a source of natural food additives. Food Chem. 2022;373(PB):131464.
95. Drevelegka I, Goula AM. Recovery of grape pomace phenolic compounds through optimized extraction and adsorption processes. Chem Eng Proc - Process Intensif. 2020;149:107845. Available from: https://www.sciencedirect.com/science/article/pii/S0255270119312474
96. Xu Y, Burton S, Kim C, Sismour E. Phenolic compounds, antioxidant, and antibacterial properties of pomace extracts from four Virginia-grown grape varieties. Food Sci Nutr. 2016;4(1):125–33. Available from: https://doi.org/10.1002/fsn3.264
97. Aquilar F, Autrup H, Barlow S. Scientific opinion of the panel on food additives, flavourings, processing aids and materials in contact with foods on a request from the commission on the use of rosemary extracts as a food additive. EFSA J. 2008;721:1–29.
98. Delshadi R, Bahrami A, Assadpour E, Williams L, Jafari SM. Nano/microencapsulated natural antimicrobials to control the spoilage microorganisms and pathogens in different food products. Food Control. 2021;128:108180. Available from: https://www.sciencedirect.com/science/article/pii/S0956713521003182
99. GRAS notification for the use of natamycin in ready-to-drink tea beverages; fruit flavored energy, sport, and isotonic drinks; and fruit flavored drinks. Food drug Adm. 2015.
100. Nisin preparation-Direct food substances affirmed as generally recognized as safe. Food and drug administration. 1988.
101. Settier-Ramírez L, López-Carballo G, Gavara R, Hernández-Muñoz P. Broadening the antimicrobial spectrum of nisin-producing Lactococcus lactis subsp. Lactis to Gram-negative bacteria by means of active packaging. Int J Food Microbiol. 2021;339:109007. Available from: https://www.sciencedirect.com/science/article/pii/S0168160520305018
102. Montiel R, Martín-Cabrejas I, Medina M. Reuterin, lactoperoxidase, lactoferrin and high hydrostatic pressure onthe inactivation of food-borne pathogens in cooked ham. Food Control. 2015;51:122–128. Available from: https://doi.org/10.1016/j.foodcont.2014.11.010
103. GRAS Notice 853, Lysozyme produced by Trichoderma reesei. food and drug administration. 2019. Available from: https://www.fda.gov/media/135212/download
104. Kummer N, Wu T, De France KJ, Zuber F, Ren Q, Fischer P, et al. Self-assembly pathways and antimicrobial properties of lysozyme in different aggregation states. Biomacromolecules. 2021;22(10):4327–36. Available from: https://doi.org/10.1021/acs.biomac.1c00870
105. Syngai GG, Ahmed G. Chapter 11 - Lysozyme: a natural antimicrobial enzyme of interest in food applications. In: Kuddus MBT-E in FB, editor. . Academic; 2019. p. 169–79. Available from: https://www.sciencedirect.com/science/article/pii/B9780128132807000116.
106. Lara-Aguilar S, Alcaine SD. Lactose oxidase: a novel activator of the lactoperoxidase system in milk for improved shelf life. J Dairy Sci. 2019;102(3):1933–42. Available from: https://www.sciencedirect.com/science/article/pii/S0022030219300141
107. Niaz B, Saeed F, Ahmed A, Imran M, Maan AA, Khan MKI, et al. Lactoferrin (LF): a natural antimicrobial protein. Int J Food Prop. 2019;22(1):1626–41. Available from: https://doi.org/10.1080/10942912.2019.1666137
108. Nieva SG, Jagus RJ, Agüero MV, Fernandez MV. Fruit and vegetable smoothies preservation with natural antimicrobials for the assurance of safety and quality. LWT. 2022;154:112663. Available from: https://www.sciencedirect.com/science/article/pii/S0023643821018168
109. Biasibetti E, Rapacioli S, Bruni N, Martello E. Lactoferrin-derived peptides antimicrobial activity: an in vitro experiment. Nat Prod Res. 2021;35(24):6073–7. Available from: https://doi.org/10.1080/14786419.2020.1821017

110. Paulino BN, Molina G, Pastore GM, Bicas JL. Current perspectives in the biotechnological production of sweetening syrups and polyols. Curr Opin Food Sci. 2021;41:36–43. Available from: https://www.sciencedirect.com/science/article/pii/S2214799321000266
111. Chattopadhyay S, Raychaudhuri U, Chakraborty R. Artificial sweeteners–a review. J Food Sci Technol. 2014;51(4):611–21.
112. GRAS Determination of erythritol for use in human food. Food and drug administratioın. 2018.
113. GRAS Notification for D-tagatose Produced by a Novel Enzymatic Cascade. Food and Drug Administation. 2020.
114. D-Tagatose Chemical and Technical Assessment (CTA). Food and Agriculture Organization of the United Nations. 2003.
115. Ahmad SY, Friel J, Mackay D. The effects of non-nutritive artificial sweeteners, aspartame and sucralose, on the gut microbiome in healthy adults: secondary outcomes of a randomized double-blinded crossover clinical trial. Nutrients. 2020;12:3408.
116. Praveena SM, Cheema MS, Guo H-R. Non-nutritive artificial sweeteners as an emerging contaminant in environment: a global review and risks perspectives. Ecotoxicol Environ Saf. 2019;170:699–707. Available from: https://www.sciencedirect.com/science/article/pii/S0147651318313368
117. Choudhary AK, Pretorius E. Revisiting the safety of aspartame. Nutr Rev. 2017;75(9):718–30. Available from: https://doi.org/10.1093/nutrit/nux035
118. Choudhary AK. Aspartame: should individuals with type II diabetes be taking it? Curr Diabetes Rev. 2018;14(4):350–62.
119. Khamise NA, Tayel DI, Helmy MW, Aborhyem S. Effect of Aspartame and sucralose artificial sweeteners on weight and lipid profile of male Albino rats. J High Inst Public Heal. 2020;50(2):87–100. Available from: https://jhiphalexu.journals.ekb.eg/article_108281.html
120. Ragi M-EE, El-Haber R, El-Masri F, Obeid OA. The effect of aspartame and sucralose intake on body weight measures and blood metabolites: role of their form (solid and/or liquid) of ingestion. Br J Nutr. 2021:1–9. Available from: https://www.cambridge.org/core/article/effect-of-aspartame-and-sucralose-intake-on-body-weight-measures-and-blood-metabolites-role-of-their-form-solid-andor-liquid-of-ingestion/F97EA712AAC19B3D5A8E98BCE78AD22E
121. Krishnasamy K. Artificial sweeteners. Weight Manag IntechOpen; 2020.
122. Musso P-Y, Lampin-Saint-Amaux A, Tchenio P, Preat T. Ingestion of artificial sweeteners leads to caloric frustration amemory in Drosophila. Nat Commun. 2017;8(1):1803. Available from: https://doi.org/10.1038/s41467-017-01989-0

Index

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