Food Microbiology and Food Safety

Sandeep Kumar Panda
Prathapkumar Halady Shetty *Editors*

Innovations in Technologies for Fermented Food and Beverage Industries

Springer

Food Microbiology and Food Safety

Series Editor:

Michael P. Doyle

More information about this series at http://www.springer.com/series/7131

Food Microbiology and Food Safety Series

The Food Microbiology and Food Safety series is published in conjunction with the International Association for Food Protection, a non-profit association for food safety professionals. Dedicated to the life-long educational needs of its Members, IAFP provides an information network through its two scientific journals (Food Protection Trends and Journal of Food Protection), its educational Annual Meeting, international meetings and symposia, and interaction between food safety professionals.

Series Editor

Editorial Board

Sandeep Kumar Panda
Prathapkumar Halady Shetty
Editors

Innovations in Technologies for Fermented Food and Beverage Industries

Springer

Editors
Sandeep Kumar Panda
School of Basic Sciences
Indian Institute of Technology
Bhubaneswar, India

Prathapkumar Halady Shetty
Department of Food Science
and Technology
Pondicherry University
Kalapet, Pondicherry, India

Food Microbiology and Food Safety
ISBN 978-3-319-74819-1 ISBN 978-3-319-74820-7 (eBook)
https://doi.org/10.1007/978-3-319-74820-7

Library of Congress Control Number: 2018937645

Printed on acid-free paper

This Springer imprint is published by the registered company Springer International Publishing AG part of Springer Nature.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Fermentation is one of the oldest food processing technologies to be used by the populations and was used for extending the shelf life of foods as well as improving the texture and sensorial characteristics of foods. Many fermented foods and beverages also helped the early civilizations to come together as well as to socialize. Many civilizations also have identified the health and nutritional benefits of fermented foods and beverages that are consumed part of the daily diet. In the recent days, there has also been increased interest in fermented foods from the point of view of health and nutrition.

Optimization of food products and scaling up is an important requirement for commercialization of any food products so that process is streamlined and the product obtained is of consistent quality. When it comes to fermented foods and beverages, process optimization is more complex due to the dynamic nature of fermentation as well as the diversity of fermentation microflora.

This book consists of 16 chapters written by experts in specific fields from seven countries, from India, South Africa, Canada, Greece, Croatia, Cameroon, and Chile. These chapters cover exhaustively innovations in starter culture; production of health beneficial fermented food products; technological intervention in beer, wine, and spirits production; marketing of alcoholic beverages; modernization of dairy plants for production of fermented dairy products; nondairy probiotics; development of automatic fermenters; and packaging technology. It includes genetic engineering for production and quality improvement of food and beverages and forecasting of the quality of the final product, specifically applications of hybrid methods combining multivariate statistics and computational intelligence, the role of consumers in innovation of novel food and beverages, and IPRS in respect to food and beverages.

We, the editors of the book, would like to acknowledge the efforts of all the authors for putting together the chapters in a very short span of time in spite of their busy schedule. We hope the book will help the readers to gain state-of-the-art knowledge in the field. Happy reading.

Bhubaneswar, India Sandeep Kumar Panda
Kalapet, Pondicherry, India Prathapkumar Halady Shetty

Contents

Chapter 1
Innovative Technologies and Implications in Fermented Food and Beverage Industries: An Overview

Lopamudra Sahu and Sandeep Kumar Panda

Abstract The chapter presents an overview of the recent developments in the different perspectives of fermented food and beverage industries. The novel innovations in starter culture, health beneficial foods and beverages, nutraceuticals, alcoholic beverages, probiotic dairy and nondairy products, and fermented meats are elucidated. Further, the technological interventions in modernization of fermenters, thermal and nonthermal food processing, genetic engineering of microbes and their applications in food industry, safe packaging technologies, and intellectual property rights are briefly described. Case studies with respect to the different innovations in food and beverages are also included for better understanding.

Keywords Fermented food · Starter culture · Probiotics · Alcoholic beverages · Fermenters · Safe packaging · Nonthermal processing · IPRS

Introduction

Fermented foods and beverages have become one of the most commonly used foods of the modern society. Fermentation techniques have been over the years improved, and novel processing techniques are being explored and implemented to meet the demands of high-quality processed foods and beverages, which would be safer, free from synthetic chemicals, and at the same time nutritious and fresh. From farm to fork, a lot of novel techniques have been employed for the optimum satisfaction of consumers and also to meet the demands of fermented food products. The modern-day food fermentation and beverage industry is highly competitive and innovative and is always in the process of upgrading, innovation, and refinement of the

L. Sahu
Department of Botany, Utkal University, Bhubaneswar, India

S. K. Panda (✉)
School of Basic Sciences, Indian Institute of Technology, Bhubaneswar, India

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_1

technology for quality improvement and new product development from diverse food sources.

The production and consumption of the fermented foods are not uniform throughout the globe; it's worthwhile to note here that the immensely assorted fermented food products are currently prevailing due to cultural diversity worldwide and preference of the consumers of a particular region. The major segments of fermented food products include dairy products, beer, wine, spirits, fermented fish, meat, and vegetables. Dairy products possess the highest stake among the other segments of fermented foods as milk and milk products are consumed by six billion people worldwide, and it provides 6–7% of the dietary protein supply in Asia and Africa and 19% in Europe. Keeping in view of the health-promoting activities of probiotic microbial strains, the demand of fermented dairy as well as nondairy products is rising rapidly. Use of probiotics in dairy products such as yogurt, cheese, ice creams, etc. are few such innovations that have increased the consumption of dairy products in leaps and bounds recently (Mishra et al. 2017). Over the years, the fermentation processes have been refined, and fermented products have been fortified with nutraceutical components keeping in mind of human's health and wellness. Various other examples such as application of encapsulation, genetic engineering, nonthermal processing for food safety and preservation, active packaging technologies, and computational intelligence techniques for forecasting problematic wine, beer, and other alcoholic beverages have been regarded as ultramodern devices and systems that have contributed colossally toward the improvement of food and beverage industries.

The present chapter is an overview of the innovations and novel techniques in fermented food and beverage industry.

Fermented Foods: Current Status and Future

Global functional food market was worth USD 129.39 billion in 2015, and it is projected to be USD 255.10 by 2024 (www.grandviewresearch.com). The demand of milk and milk products is in an increasing trend for the last three decades. Global milk production has increased by more than 50%, from 500 million tons in 1983 to 769 million tons during 2013. In South Asia, consumption of milk and milk products is projected to increase by 125% during 2030. Similarly, beer possesses the largest market share among the alcoholic beverage industries with a global production of 1.96 billion hectoliters. China is the largest producer of beer (46.54 million kiloliters) in 2013 followed by the US (22.43 million kiloliters) and Brazil (13.46 million kiloliters) in the same year (http://www.worldatlas.com). The global wine production was 259 million hectoliters in 2016, and the consumption was 240 million hectoliters. The top wine producers are Italy (48.8 million hectoliters), France (41.9 million hectoliters), and Spain (37.8 million hectoliters) (www.bkwine.com). Commercially important fermented foods and the major producing regions are elucidated in Table 1.1.

Table 1.1 Commercial fermented foods and the major producing regions

Products	Microorganisms used	Type of fermentation	Major producers	References
Wine	*Saccharomyces cerevisiae*	Submerged	Italy, France, Spain	Mishra et al. (2017)
Beer	*Saccharomyces cerevisiae*, *Saccharomyces pastorianus*	Submerged	China, USA	Stewart (2016)
Whiskey	*Saccharomyces cerevisiae*	Submerged	France, Scotland, USA, Canada	Walker and Hill (2016)
Yogurt	*Streptococcus thermophilus*, *Lactobacillus delbrueckii*	Submerged	France, Ireland, Canada, USA	Han et al. (2016)
Cheese	*Lactococcus*, *Lactobacillus*, *Streptococcus* sp., *Penicillium roqueforti*	Solid-state fermentation	Germany, the Netherlands, France, USA	Mishra et al. (2017)
Acidophilus milk	*Lactobacillus acidophilus*	Submerged	North America, Europe, Asia	Yerlikaya (2014)
Sauerkraut	*Leuconostoc* sp., *Lactobacillus brevis*, *Lactobacillus plantarum*	Solid-state fermentation	Europe	Swain et al. (2014)
Fish sauce	Lactic acid bacteria (halophilic), *Halobacterium salinarum*, *Halobacterium cutirubrum*, *Bacillus* sp.	Submerged fermentation	Thailand, Korea, Indonesia	Lopetcharat et al. (2001)
Fermented meat	*Lactobacillus sp,. Micrococcus sp., Staphylococcus sp.*	Solid/submerged fermentation	Europe	Holck et al. (2015)

Starter Cultures for Fermented Foods

In indigenous and traditional fermentation process, food processing was carried out without knowing the principles and biochemistry of the microorganisms involved; hence, the final product was unpredictable and with varying quality. Gradually, the knowledge of the involvement of microorganisms and their mode of functioning influenced the food scientists to use specific microorganisms for food fermentation (Mishra et al. 2017). Starter culture is defined as the strain of microorganism selected with stable features to produce desirable characters of food under controlled conditions (Wakil et al. 2014). The common groups of microorganisms involved in food fermentations are bacteria, yeasts, and molds. Starter cultures are classified as single strain (contains only one strain of same species), multi-strains (three or more single strains of defined mixture), and mixed strains (contains unknown strains) (Mishra et al. 2017). The most important bacteria in the fermentation of foods are the Lactobacilli, which have the ability to produce lactic acid from carbohydrates. Other important bacteria are the acetic acid producing *Acetobacter* (mainly from fermentation of fruits and vegetables) and *Bacillus* (from fermentation of legumes) species. The beneficial yeasts in terms of desirable food fermentations are from the *Saccharomyces* family, especially *S. cerevisiae*. Yeasts play an important role in the food industry as they produce enzymes that result in benevolent biochemical reactions imparting typical flavor and aroma in wine, beer and ethanol, and leavening of bread. The lactic acid bacteria (LAB) are, however, the most commonly found microorganisms in fermented foods. Their crucial importance is associated with physiological features such as substrate utilization, metabolic capabilities, and probiotic properties. Their common occurrence in foods coupled with long historical use contributes to their acceptance as GRAS (generally recognized as safe) for human consumption. Various LAB have been isolated from different fermented foods. Their functions during or after food fermentation have gradually been elucidated.

Innovations for Better Health and Nutrition

Fermented foods have long been used as source of nutrition. The demand for fermented foods and the newly conceptualized functional foods are in the rise owing to its potential health benefits. These foods have been explored for their various medicinal properties in the recent past such as antihypertensive (Ahren et al. 2014), antidiarrheal (Kamiya et al. 2013), blood glucose-lowering benefits (Oh et al. 2014), and antithrombotic properties (Kamiya et al. 2013), to name a few. The health benefits of the fermented foods can be accounted to the presence of bioactive compounds in the form of phytochemicals such as phenolics, flavones, fatty acids, and saccharides along with vitamins, minerals, and amino acids in substantial quantities as compared to their nonfermented forms (Rodgers 2008; Rodriguez et al. 2009; Capozzi et al. 2012; Sheih et al. 2014; Xu et al. 2015). Fermented foods are known to accumulate bioactive compounds during the processing of the food which are naturally absent or present in very low amounts in the unprocessed counterparts. For instance, red ginseng roots contain bioactive compounds such as saponins (ginsenosides) and nonsaponins, while the fermented ginseng roots have shown increased levels of saponins (Oh et al. 2014). These saponins are known to regulate the blood glucose and insulin levels (Oh et al. 2014). Fermented soybeans, a major staple food consumed in Korea, China, Japan, Indonesia, and Vietnam, has been reported for exhibiting antidiabetic effects. Such properties in soybean are partly due to both quantitative and qualitative changes in the small molecules during the processing of the fermented product (Kwon et al. 2010). Dickerson and his colleagues have demonstrated defined antioxidant and immune-modulating potentials of fermented papaya (Dickerson et al. 2015). Certain foods and beverages fermented with specific LAB strains are known to represent immune regulation and antiallergenic properties (Nonaka et al. 2008). Recently, metabolomics in conjunction with microbial ecology and genomics are scientific tools intended toward finding out the disease-fighting properties of the bioactive compounds and discovering novel ones from fermented foods.

Technological Advances in Novel Food Processing

Fermentation is an age-old food processing technology, the knowledge of which has been passed on to successive generations (Caplice and Fitzgerald 1999; Adebo et al. 2017a). In lieu of the rising population, conventional fermentation techniques are impossible to cater to the needs of the consumer demands, and modern-day technology can ensure fast delivery of desired fermented foods while maintaining consistency in superior quality, sensory attributes, and nutritional benefits (Todorov and Holzapfel 2015; Adebiyi et al. 2017; Adebo et al. 2017a, b). Novel food processes are being adapted in food and beverage industries with the advent of emerging new age technologies and scientific research in food science. Efficient use of various novel innovations such as co-culture, thermophilic fermentation, molecular tools, genetic engineering, mutant selection, and recombinant DNA technologies have made it possible to design and construct tailor-made starter cultures that perform better than those found naturally. For instance, a novel beverage was obtained by using mixed starter cultures, viz., *Saccharomyces cerevisiae* and *Pediococcus acidilactici*, *S. cerevisiae* and *Lactobacillus acidophilus*, *P. acidilactici and L. acidophilus*, and *S. cerevisiae*, *P. acidilactici*, and *L. acidophilus* (Santos et al. 2014). With the rising competitiveness in the fermentation industry, innovations for the processing technologies for the delivery of novel, innovative, high-quality foods for the seething consumers of fermented foods around the world have taken a major breakthrough.

Advances in Thermal and Nonthermal Applications

The thermal processes are intended toward improving the shelf life of the fermented products, annihilating the pathogenic microorganisms, increasing the metabolic activities and production of enzymes, as well as shortening the fermentation process (Melikoglu 2012; Siefarth et al. 2014). Few thermal processes include ohmic heating, radio frequency, and microwave heating. However, thermal processing affects the original biochemical, texture, and sensorial properties of fermented foods. The most preferable novel nonthermal processes used in the fermentation industry are high-pressure processing (HPP), high-pressure carbon dioxide (HPCD), ultrasound, ultraviolet radiation (UV), gamma (γ) irradiation, microwave irradiation, and pulsed electric field (PEF) (Gupta and Abu-Ghannam 2012). FDA (Food and Drug Administration, USA) has approved the use of γ-irradiation for food processing, which is obtained from cobalt-60 or cesium-137 and UV radiation for surface sterilization. Preservation of food through ionizing radiation with a dose up to 7 kilogray is regarded as safe by WHO and is accepted by countries such as the USA, France, Netherlands, Canada, etc. Likewise, UV radiation is a nonionizing ray that either kills bacteria or inhibits the bacterial reproduction by interfering in the DNA replication through formation of thiamine diamers in the chromosome (US Food and Drug Administration 2007). HPP is applied in a range of 100–800 MPa in different temperatures to both solid and liquid food products to ensure sterilization.

It destroys the cytoplasmic membrane of the contaminant microorganism. Loss of solute, enzyme inactivation, and protein coagulation are the main reasons of cell death. HPCD is regarded as an environment-friendly and energy-saving process as it uses less pressure and CO_2 is nontoxic. PEF is applicable to liquid and semiliquid samples, food material is placed between two electrodes, and pulses of high electric field (1–50 kV/cm) for very short periods (2 μs–1 ms) are applied for sterilization (Barbosa-Canovas et al. 2001). Apart from these nonthermal processes, various natural antimicrobials are added in the form of extracts from herbs, spices, and vegetables. Extracts of seaweeds, capsicum, and green tea inhibit the growth of *Salmonella* sp. Essential oil of herbs like rosemary, basil, ginger, and sumac contains compounds such as carvacrol, citral, thymol, eugenol, and citric acid well studied for their antimicrobial activity (Gupta and Abu-Ghannam 2012). The nonthermal processes basically accelerate the chemical reactions during the processing such as oxidation, polymerization, condensation, and esterification, augmenting fermentation rates and monitoring the fermentation process, and pasteurization (Oey et al. 2008; Tao et al. 2014; Mirza-Aghayan et al. 2015). Along with the thermal and nonthermal processes, other techniques which are being explored in the recent past include nanotechnology and metabolomics.

Nanotechnology

Nanoscience and nanotechnology have huge potential in a number of fields including medicine, cosmetics, agriculture, and food. The scientific significance of nanotechnology lies in its ability to study particles at nanoscale (1–100 nm) which are expectedly biologically more active than the larger-sized particles of the same constituent. Though the fermented foods are rich in nutrients and other active components, yet these may be lost during processing of the foods. Nanoemulsion formations have been used successfully to safeguard nutraceuticals against degradation, providing better stability, and augment the bioavailability and safe delivery of functional ingredients to potential consumers. Another application of nanoscience is nano-sensors which are being employed for effectively monitoring and evaluating quality parameters of fermented foods. These include quantum dots, biosensors for detecting the pathogenic and deleterious microorganisms that cause spoilage of food.

Food Metabolomics

There have been conscious and intensified efforts for development and delivery of functional foods which confer additional health benefits (Adebo et al. 2017a). Fermented foods are unique in their composition with many identified and characterized components and unidentified ones. The analysis of these components can open up new avenues for the food fermentation industry. With the introduction of "omics" in food industry, food metabolomics or foodomics as it is called has become possible to characterize the complete profile both quantitatively and qualitatively of the food metabolome. With such sophisticated analytical equipments, details about the fermented foods like their composition and metabolic interactions can be identified that could help in correlating with the functional aspects. Important factors that establish the selection of steps include the type of study (targeted or untargeted), solid or liquid form of sample, and the instruments used [gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR), etc.]. The process of metabolomic analysis includes sample preparation, extraction, data acquisition, and data analysis (Adebo et al. 2017a). Metabolomic study was conducted to observe the metabolic changes occurring during the fermentation of tea (Lee et al. 2011; Tan et al. 2016). Proton NMR, UPLC-Q-TOF-MS, and PCA were applied to study the fermentation pattern of tea fermentation, and the combination of analyses revealed that caffeine epicatechin, epigallocatechin, caffeine, quinate, theanine, and sucrose decreased gallic acid and increased glucose level. Successful fermentation patterns and metabolomics have been studied and reported for other traditional fermented foods such as kimchi (fermented vegetables), koji (fermented rice), and doenjang (fermented soybean) (Adebo et al. 2017a). A thorough understanding of the processes and parameters and utilizing this technique would aid in improving product quality and reduce product costs.

Alcoholic Beverages

Although there are several alcoholic beverages consumed throughout the globe, most of those are regional and not known to the other parts of the world. The globally accepted alcoholic beverages are beer, wine, and spirits such as whiskey, vodka, gin, and rum. Beer is the largest selling alcoholic beverage followed by distilled spirits, and it is the third most consumed drink after water and tea (Panda et al. 2015). Currently, craft breweries and distilleries are becoming more popular and getting more preference for their innovative strategies during production. Craft breweries produce seasonal beers with different flavors and alcohol levels such as strong beer from caramelized malt during winter and lighter beers with citrus flavor during summer (Kellershohn and Russell 2015). Similarly, the craft distilling units are going up in the last few years as they offer new aroma and flavor. Further, unique and novel packaging presentations are very attractive in craft breweries and distilleries. As craft breweries and distilleries operate in small batches, they have the opportunity to experiment different production batches with varieties of raw materials and different fermentation techniques. Nowadays, newer substrates of tropical origin are used for wine production with nonconventional organoleptic characteristics. Tropical wines have been prepared from fruits like mango (Reddy and Reddy 2005), tendu (Sahu et al. 2012), jackfruit (Panda et al. 2016), litchi (Kumar et al. 2008), bael (Panda et al. 2014a), sapota (Panda et al. 2014b), etc.

Some genetically modified (GM) yeast strains are developed to reduce the fermentation period; some generate desirable flavors, and some use residual sugar left in beer in contrast to non-GM strains. Similarly, in the case of wine fermentation specialized yeast, *Saccharomyces cerevisiae* ML01 was genetically modified to suppress the production of biogenic amines, toxic substances that are produced during wine fermentation (Husnik et al. 2007). Another recombinant *S. cerevisiae* strain is known to reduce the formation of ethyl carbamate, a carcinogen produced during wine fermentation (Dahabieh et al. 2009). Nowadays, biomolecular methods are adopted to study the fate of microorganisms in alcoholic beverages. Characterization of specific starter culture and undesirable yeast or bacteria is being carried out by fast molecular methods such as PCR, RAPD-PCR, PCR-TTGE, PCR-DGGE, short tandem repeats, etc. (Comi and Manzano 2008). Although GM yeasts with efficient alcohol production and tolerance capacity are developed, low preference of consumers for GMO makes it difficult for wide application; hence, hardly any reputed brewery or distillery use GM yeast as starter culture.

Advances in Probiotics, Prebiotics, and Nutraceuticals

The term functional food was first coined in Japan, and it refers to processed foods containing ingredients that have specific health benefits in addition to nutrition (Kaur and Das 2011). There are many types of functional foods. Nutraceuticals are dietary supplements that are also called functional foods. They are produced from foods and sold in the medicinal form of capsule, tablet, powder, and solution (Ali et al. 2009). Nutraceuticals have been claimed to have a physiological benefit or provide protection against diseases including cardiovascular agents, antiobese agents, antidiabetics, anticancer agents, immune boosters, chronic inflammatory disorders, and degenerative diseases. The widely adopted definition of probiotics is "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2001; Fuller 1989; Panda et al. 2017). There are two other important terms, prebiotics and synbiotics. Prebiotics are defined as the complex food components added with the probiotic to promote its growth and activity. Synbiotics are combinations of probiotics and prebiotics designed to improve the survival of the ingested microorganisms and their colonization of the intestinal tract (de Vrese and Schrezenmeir 2008).

Along with addition of probiotic starter cultures in the manufacture of fermented dairy products, it is of quite interest due to its therapeutic and prophylactic properties as these bacteria improve the population of the beneficial intestinal microflora. Two of the most important probiotic genera are *Lactobacillus* and *Bifidobacterium*, while few other species of interests are from the genera *Pediococcus*, *Enterococcus*, and *Lactococcus*. However, selection is preferably based on their GRAS (generally regarded as safe) status, prolonged safe use in foods, nonpathogenic character, stability in acid and bile, etc. (Doder et al. 2013). The survival of the probiotics during processing and storage of fermented products requires a lot of optimization of technological parameters. Prebiotics in fermented food stimulate the gut microflora, besides providing textural attributes to the foods (Saad et al. 2013). Probiotic-fermented soymilk supplemented with the fructooligosaccharide (FOS), inulin, and pectin increased the angiotensin I-converting enzyme inhibitory activity thereby enhanced the antihypertensive effect (Yeo and Liong 2010). Recently, Rastall and Gibson (2015) reviewed the role of prebiotics in promoting the growth of beneficial microbes and improving the intestinal health. Different starter cultures either single or in combination such as the bacteria, yeasts, and molds offer a wide range of different health prospectus with new bioactive components. Milk-derived bioactive peptides such as lactoferrin, certain free amino acids, micronutrients, sphingolipids, or exopolysaccharides have antihypertensive, antimicrobial, antioxidative, immune-modulatory, and mineral-binding properties (Korhonen 2009; Samaranayaka and Li-Chan 2011; Udenigwe and Mohan 2014).

Innovative Probiotic Dairy Products

The common fermented dairy products are yogurt, cultured buttermilk, acidophilus milk, *zabadi*, *labneh*, *biogarde*, *bifighurt*, *dahi*, etc., mostly fermented by mesophilic and thermophilic lactic acid bacteria while *kefir*, acidophilus-yeast milk, are the alcohol-lactic fermented ones. Similarly, innovations in cheese processing have made its availability in market with different flavors and aroma. The most commercially adopted cheeses are cheddar, Camembert, and Roquefort (Law and Hansen 1997). Such fermented dairy products have additional functional ingredients like prebiotics and probiotics, which have pronounced nutritive characteristics and functionality. Plant-based antioxidants have contributed immensely toward protecting the cells from oxidative stress and severe diseases (Aliakbarian et al. 2012; Halah and Nayra 2011). Production of such novel functional fermented dairy products, for example, the fermented milk enhanced with phenolic extracts from olive and grape pomace (Aliakbarian et al. 2015) using co-culture of two different microorganisms *Streptococcus thermophilus* and the probiotic *Lactobacillus* sp. and supplementation of fermented milk with neem (*Azadirachta indica*) (Shori and Baba 2013), has shown significant antioxidative activity. Manufacturing a probiotic product basically entails an ideal starter culture that can reach the target site and act in the gastrointestinal tract of the host, evaluation of the starter culture with respect to viability and functionality during processing steps, and finally successful integration of the probiotic culture into the product. Their functions have been predicted mostly by in vitro tests, but recent advancement in molecular biology has made it possible to predict the functional attributes through omic approaches. Culture selection involves the assessment of a culture's safety, efficiency to reach the colonization site and probiotic potential, considering the health benefits exerted to the consumer. Recent advances in molecular biology have allowed the prediction of its various properties such as antagonistic property against pathogens, production of enzymes, colonization at the target site, antibiotic resistance (Varankovich et al. 2015), etc. Probiotic cultures have also been bioengineered to confer it with the desired properties or targeted delivery of bioactive molecules for prevention and/or treatment of various diseases, viz., antidiabetic activity (Ma et al. 2014), antitumor activity (Wei et al. 2016), and antimicrobial activity (Volzing et al. 2013), to name a few. Such studies are expected to increase in the future while elevating the selection criteria for the characterization of potential probiotic strains.

Innovations in Fermented Fruits and Vegetables

The health benefits of probiotics on human beings have been quite visible in view of the market popularity of the fermented milks, yogurts, and fermented meats. But, increasing fondness of consumer's vegetarianism, consumption of nondairy-based products of highly nutritional value, health-promoting and rich flavor beverages, for example, market of fermented juices, smoothies, and yogurt-like products, are accelerating. Food companies, intimidated by the market growth and high margins, have been investing in the development of new nutrition-modified and functional products (Khan et al. 2014). Though dairy products have a huge market, the nondairy beverages market is also mounting, and it is expected to have an annual growth rate of 15% during the next few years (Marsh et al. 2014). Several nondairy probiotic products such as fermented fruits and vegetables (Montoro et al. 2016; Park and Jeong 2016; Neffe-Skocinska et al. 2017) have already made their presence in the market. Lactic acid-fermented fruit and vegetable juices from carrot, potato, beetroot, watermelon, sapodilla, lettuce, lemon, pepper, parsley, cabbage, spinach, tomato, gourd, pomegranate, black currant, orange, grapes, apple, pear, and cashew apple are few vegetables and fruits used in the production of the popularly called as "lactojuices." Lactojuices and lactopickles were successfully prepared from anthocyanin and β-carotene-rich sweet potatoes with acceptable biochemical and organoleptic attributes (Panda and Ray 2016). These nondairy-based beverages have gained considerable market value and consumer acceptance, as they are perceived to be healthy and refreshing. The manufacturing of fermented vegetable juices usually involves a spontaneous fermentation by autochthonous microbiota or by selected starter cultures added into raw vegetables and mild heat-treated vegetables by starter cultures. Therefore, studies are mainly focused on the design of starter cultures. Most probiotics starter cultures belong to *Bifidobacterium* and low GC % lactic acid bacteria. Yeast is a significant part of the distinguishing microbiota of several traditional fermented products associated with health benefits. *Saccharomyces boulardii* is the most recognizable probiotic yeast. Fruits and vegetables are naturally rich source of beneficial nutrients, and their internal structures (like intercellular spaces, stomas, capillaries tissues lesions, etc.) favor the probiotic integration and protection. Microbial viability and its functionality are dependent on the strain used for the starter culture and also the substrate used. *Lactobacillus* sp. and *Bifidobacterium* sp. have been reported to survive longer in orange and pineapple juice than in cranberry juice (Sheehan et al. 2007). Probiotic bacterial strains may also be isolated from raw fruits and vegetables other than the human gut and can be alternatives for novel probiotics, which have the capability of adhering to the gut epithelium and exerting beneficial effect on the health of the consumer (Vitali et al. 2012). The successful probiotication of numerous fruit and vegetable juices with lactic acid bacteria and *Bifidobacteria* has been reported. For instance, the addition of yeast autolysate (e.g., spent brewer's yeast) into the juices increases the lactic acid bacteria numbers during the process of fermentation thereby reducing the time of fermentation. One of the best examples of an innovative vegetable-based fermented beverage is smoothies. A novel protocol for the manufacture of fermented red and green smoothies was formulated, which included white grape juice and *Aloe vera* extract mixed with red cherries, blackberries prunes, and tomatoes plus green fennels, spinach, papaya, and kiwi by Di Cagno et al. (2011).

Technological Advances in Fermented Meat Products

Fermented meat is produced both by traditional methods and industrial processes. Europe is the largest producer of fermented meat products (Holck et al. 2015). They are mainly consumed for their rich sources of proteins, fat, essential amino acids, minerals, and vitamins (Biesalski 2005). For the production of more consistent, economic, and consumer-friendly fermented foods, research studies have focused on the identification of genetically improved starter cultures and the role of enzymes in the development of the physical and sensory characteristics of fermented meats. A lot of external and internal factors such as quality of meat, fatty tissue and additives, salting/curing, casing, sausage batter preparation, brine, microclimate, and starter cultures added to the product affect the production of fermented sausages and cured meat. All these conditions affect both the quality and safety of the final processed product. However, safety of the final processed food is the key consideration that has led to the emergence of novel technologies that would impart stability and improved safety while maintaining the inherent properties of the products. Safety is also of critical importance to consumers to ensure protection of toxicological and microbiological hazards. Nonthermal processing technologies, such as high hydrostatic pressure (HHP) processing, pulsed electric field (PEF), X-ray irradiation, pulsed ultraviolet (UV) light, and power ultrasound, are being considered across a wide spectrum of meat products for decontamination purposes and process optimization that could be beneficial to fermented meat processing.

Molecular Biology for Study of Microbiome of Fermented Foods

Complex fermentation involves a defined/undefined starter culture to ferment a substrate to a product such as cheese, malolactic wine, fermented seafoods, etc. (Alkema et al. 2016). It is quite important to study the microbiome during different intervals of fermentation for maintaining safety and quality of the final product. *Iru*, natural fermented African locust bean (*Parkia biglobosa*) seeds (16 samples of different regions), was subjected to both culture-based genotyping and culture-independent genotyping to assess the bacterial composition (Adewumi et al. 2013). DNA sequencing of the 16S rRNA genes obtained from PCR-DGGE identified species related to *Bacillus subtilis* as consistent bacterial species in the *iru*, whereas other main bands were identified as close relatives of *Staphylococcus vitulinus*, *Morganella morganii*, *B. thuringiensis*, *S. saprophyticus*, *Tetragenococcus halophilus*, *Ureibacillus thermosphaericus*, *Brevibacillus parabrevis*, *Salinicoccus jeotgali*, *Brevibacterium* sp., and uncultured bacteria clones. Similarly, 14 fermented samples of pearl millet slurries were collected of which 137,469 sequences of bacterial 16S rRNA gene amplicons were characterized (Humblot and Guyot 2009). Except for a few *Proteobacteria*, the rest bacterial sequences were attributed to cultivable bacteria. The bacterial sequences were allocated to four phyla, *Firmicutes* (which includes the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella*) representing the highest diversity, followed by *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*, which were found only in the slurries prepared in traditional production units. Most of the *Firmicutes* varied during the course of fermentation. In another study, the microbial diversity of Brazilian kefir grains by PCR-DGGE and pyrosequencing was analyzed (Leite et al. 2012). PCR-DGGE study showed *Lactobacillus kefiranofaciens* and *L. kefiri* are the major dominating bacteria. Pyrosequencing produced a total of 14,314 partial 16S rDNA sequence reads. *Firmicutes* was the major phyla, and *Lactobacillus* represented 96% of the sequences. Microbiome of *pu-erh* (fermented tea of China) was studied for both the process of preparation, i.e., traditional raw fermentation and faster ripened fermentation. Fungal and bacterial communities were characterized using rDNA amplicon sequencing. Three-hundred ninety fungal and 629 bacterial operational taxonomic units were identified, and it was observed that fungal diversity drops and bacterial diversity rises due to raw and ripened fermentation and fungal and bacterial composition changes significantly between fresh leaves and both raw and ripened *Pu-erh*.

Modernization of Fermenters for Food and Beverage Industry

Fermenter or bioreactor is an apparatus used to process food and other bioproducts biologically in a controlled environment. Fermenters are specially designed keeping in view of the finished products and the raw materials used. Basing on the feeding of the nutrients, bioreactors are grouped into continuous, fed-batch, and batch form of operation. Stainless steel is the preferred material for building of large-scale fermenters in food and beverage industries owing to its corrosion resistance property (Jagani et al. 2010). Various critical engineering aspects are involved in the bioreactor designing and are frequently modified and modernized to upgrade and refine the quality and productivity of the final product. The important features of a bioreactor are vessel shape, height to diameter ratio, agitation and agitator type, aeration, and baffles (Jagani et al. 2010). Further, modern devices added to the fermenters are self-cleaning microsparger, mechanical foam breaker, temperature, pH and pO_2 probe, valves, steam traps, and sampling ports. The most significant innovation is automation of fermenters or bioreactors by coupling them with computer. Computer has three important roles in food fermenters as postulated by Nyiri in 1972: (a) logging of process data, performed by data acquisition, which has hardware and software components; (b) data analysis, data reduction is executed by a data analysis system based on series of mathematical equation; and (c) process control, signals from the computer are received and a program directs for accurate functioning of pump, valve, and switches. The details about the modernization of fermenters of food and beverage industries are elaborated in Chap. 11.

Recent Developments in Packaging Technologies for Food and Beverages

Packaging is indispensable for maintaining the quality, freshness, sustainability, and safety of the produce as well as the processed products. From the initial basic paper and carton packaging to the modern-day vacuum processed packaging, it has come a long way with the changing lifestyles. The food packaging industry is evolving continuously owing to the modern-day challenges (Realini and Marcos 2014). Earlier packaging of food materials includes metal, glass, paper, plastics, foil, wood crates, or burlap but currently, microwaveable packages, single-serve packs, and zippered packages are more preferable by consumers (Ferrante 1996). The packaging industry is improvising technically and reducing environmental stress due to packaging waste and food loss while ensuring a fresher and contamination-free, safer, and increased shelf life of foods and beverages. Recent advances in active packaging which involves oxygen, carbon dioxide, ethylene scavengers, and moisture control agents, viz., antimicrobials and antioxidants, nano packaging, aseptic packaging, packaging mechanisms controlling volatile flavors and aromas, advancements in food packaging distribution such as radio frequency identification (RFID) and electronic product codes, etc., have changed the scenario of modern-day food packaging. Active packaging is intended to absorb the undesirable substances liberating from the packaged food for inhibiting its deterioration of foods and maintaining freshness (Wyrwa and Barska 2017). Smart packaging uses chemicals and biosensors for monitoring of quality and safety of food. Thus, online quality control system should be developed with highly efficient sensors integrated in the packaging to cross-check the quality of the product in terms of weight, volume, color, appearance, and biochemical composition (Kuswandi et al. 2011). An increasing concern in environmental pollution has led to bio-formulations based on food packaging which has a huge demand in the market. Details of the above processes are dealt in details in Chap. 14.

Consumers' Role in Innovation of Novel Food and Beverages

One of the main aims for the technological developments and innovations in the food industry has been not only to meet the consumers' demands for such novel and healthy foods but also the consumers' acceptance. Some technology-based innovations have been easily adopted, while some are easily rejected like those of the genetically modified foods (GMFs) in Europe and food irradiation (Ronteltap et al. 2007). So, the long-term success of a new food product largely depends upon the consumer's perception and acceptance. Market research and sensorial analysis are the common tools used.

Innovation is basically the implementation of an idea which is feasible, economically viable, and translated into a successful product, and this involves a myriad of tasks such as the marketing, technology, partnership, commercialization, safety issues, and risk factors. Again, with growing awareness and preferences of consumers for healthy foods, their roles in the innovation practices are essential. A lot of factors influence the consumers' acceptance of a novel food such as their involvement in the product processing; cultural, social, and lifestyle factors; long-term effects of novel technologies such as risks and concerns on the human health; etc. Therefore, it becomes important to assess the consumers' perception toward development and marketing of products that would aid in developing effective food marketing strategies (da Silva et al. 2014).

As consumers are becoming more enlightened due to the advancement of technology, potential health benefits of functional foods and nutritional supplements are being regularly assessed by the consumers. Though scientific studies prove these functional foods to be safe, yet consumers are skeptic about their long-term health risks. Keeping all these concerns, a large section of the human population are seeking for minimal processed food products which are clean and green such as artisan foods and organic foods.

Genetic Engineering for Higher Production and Quality Improvement

Genetically modified foods are obtained by incorporating or modifying a targeted gene of an organism, which may be a plant, animal, or microorganism. The gene modification is generally accomplished in three ways: (a) direct transfer of DNA through microparticle bombardment, (b) transfer of DNA through bacterial vehicles (T-DNA), and (c) editing of genomic DNA (gene knockout or gene addition through CRISPR-Cas 9 system) (Zhang et al. 2016). Application of the latest molecular tools in the fermentation industries has paved the path for better product yields, superior quality products, and manufacturing of novel foods. For instance, transcriptomics tools have assisted in elucidating the molecular mechanisms of the metabolic transformations of fermenting microorganisms. Of late, the complete genome profiling of important fermenting yeasts and bacteria species has been included in the public databases; the genomic databases have enabled the study of gene expressions under different conditions providing new insight into important metabolic processes (Bokulich and Mills 2012). Microarrays have helped to study the stress factors in a fermentation process affecting the transcriptional response for laboratory or industrial wine, lager brewing and baker's yeast strains (Shima et al. 2005; Tai et al. 2007; Rossignol et al. 2006), gene expression dynamics during the various stages in fermentation of synthetic media or natural substrates (Penacho et al. 2012), and transcriptional variation concerning the diverse strains and mutants (Bartra et al. 2010). In addition to yeasts, LAB also have industrial relevance, as they can provide the key flavor, desirable texture, and preservative qualities to a variety of fermented foods, such as sourdoughs, dairy products, and fermented sausages (Klaenhammer et al. 2007). Gene expression microarray has been quite helpful in analyzing the critical gene groups involved in relevant metabolic and functional activities (Hufner et al. 2008). Next-generation sequencing or RNA-seq has taken gene expression analysis to a higher level, improving the possibilities for investigating novel aspects in the transcriptomes of fermenting microorganisms (Solieri et al. 2013). Profiling of microbial communities and elucidation of the molecular mechanisms of the metabolic processes and pathways involved in fermented food ecosystems are very important for food science and the industry. In the above perspective, the new omics approach, known as metatranscriptomics (analyses of expressed genes from complex metagenomes), will significantly foster our knowledge about microbial behaviors in food ecosystems and utilize them for quality improvement and higher production of food and beverages.

Genetically Modified (GM) Foods: Pros, Cons, and Legislation

The global population is rising and is expected to reach 9.7 billion in 2050 from 7.35 billion in 2015, and currently 795 million people of the world are undernourished (FAO 2016). Further, the annual rate of increase in crop yield is within 1.7%, whereas to meet the global demand, it should be 2.4%. Keeping in view of the increasing global population and insignificant rise in growth rate of food crops, GM foods with higher yield and quality of consumers' preference are debatable. Various agronomic benefits include an additional rise in the global crop production in the limited farming area (corn, 274 million tons; soybeans, 138 million tons; cotton lint, 21.7 million tons; canola, 8 million tons) (Brookes and Barfoot 2014), economic gains, and reduced usage of risky pesticides and herbicides (James 2013). Other innovative products include the enrichment of vitamins (A, C, E) and amino acids to food products and additives. Golden rice (rich in vitamin A), amflora (potato rich in amylopectin for industrial use), and methionine-enriched sweet lupine are prominent examples (Zhang et al. 2016). Other important achievements are "FlavrSavr" tomatoes (suppression of polygalacturonase) and "Aqua Advantage," first genetically modified animal (fast-growing salmon) approved by FDA for consumption.

GM microorganisms are designed to overproduce a targeted product or to inhibit the generation of undesirable metabolites. Trehalose, a disaccharide (formed by conjugation of α-glucose molecules), is known for accumulation of ~30% of yeast cells in thermal stress which is a pressing problem for the bakery industry. To overcome the aforesaid problem, a new yeast strain was obtained by disrupting the trehalose (*ATH1*) gene and with tolerance to stress such as dehydration, freezing, and toxic levels of ethanol (Kim et al. 1996). In another study, yeast hybrids were generated using strains of a domesticated, flocculent *Saccharomyces* and *S. eubayanus* that showed accelerated fermentation and higher ethanol production (5.6%) as compared to their parental strains (4.5%) (Krogerus et al. 2015). Similarly, a recombinant *S. cerevisiae* strain was constructed that possess accelerated maturation and high glucoamylase activity, but no heterologous DNA sequences, bacterial sequences, or drug-resistant genes were incorporated to the new yeast; hence, the new strain may be regarded as safe for application in breweries (Wang et al. 2010). Incorporation of β-glucosidase genes from *Aspergillus*, *Hanseniaspora*, or *Pichia* genera to wine yeasts resulted in new yeast strains with potential of producing high flavor compounds like terpenoids in must and wort (Verstrepen et al. 2006). Folate is an important B group vitamin known to protect against cancers, cardiovascular diseases, and risk of neural tube defects. A few LAB are known to synthesize folic acid. Wegkamp et al. (2003) cloned five essential folate biosynthesis genes from *L. lactis* MG1363 into the pNZ7017 vector. Further, the vector was incorporated into the nonproducing folate strain *L. gasseri* ATCC 33323, which showed capacity of producing folate.

However, despite of several advantages, various health and ecological hazards like competition with natural species associated with GM foods have created a dilemma among the consumers. Allergic reactions have been reported with the consumption of “StarLink” maize and transgenic soybeans. Different countries have different regulations regarding the GM foods. Most countries allow the production and marketing through their own legislation and regulation. However, countries like Russia, Norway, the Netherlands, and Israel don’t permit the GM foods for production and sale in their jurisdiction (Csutak and Sarbu 2018). Although, China, France, South Korea, and New Zealand permit GM foods but the adaptation is very slow. China has not enforced any specific laws for GM foods. The Ministry of Agriculture and the General Administration of Quality Supervision, Inspection and Quarantine currently deals with GM foods. GM papaya is permitted for cultivation and propagation in China (Wong and Chan 2016). Similarly, the USA doesn’t have any specific federal law for GM foods and the regulations are liberal. Agencies like the US Department of Agriculture (USDA), the Environmental Protection Agency (EPA), and the FDA (Food and Drug Administration) are the controlling institutions regarding GM food policies and approval. European Union (EU) legislation is very stringent about the use of GMO in foods, whereas the USA and Canada are comparatively liberal. The EU considers GM microorganisms obtained from uncontrolled genetic changes such as random mutagenesis, dominant selection, adaptive evolution, natural conjugation, and transformation, but the microbes developed through recombinant DNA technology is generally rejected for application in food and beverage industries (Csutak and Sarbu 2018). It is mandatory to label "GM foods" for marketing the products in the jurisdiction of the EU. Exclusive legislation of GM foods in most of the countries is in the horizon and the present form is very confusing.

Intellectual Property Rights (IPR) in Respect to Food and Beverages

With the ever-growing population and the environment changes, there is depletion of available food and the natural resources. This has necessitated scientific research on the existing practices and replacing them with innovative ones for higher production of foods with potential health benefits. The fast-changing demands of the consumers' needs have emphasized for development of novel products in the food and beverage industry with which the role of intellectual property rights (IPR) has emerged to safeguard the companies' innovations and ideas from infringement. The various types of IPR protection in food and beverage industry include patents, copyrights, trademarks, industrial designs, trade secrets, and geographical indications. All these have been covered in details in Chap. 16.

Conclusion and Perspectives

The current functional food and beverage market is as such too huge and consists of wide ranges of food products, i.e., from alcoholic beverages to fermented meat and from probiotics to fermented fruits and vegetables. It is estimated that the growth of global functional food market is at 6% CAGR during 2011–2015. The global craft beer market is expected to reach USD 502.9 billion by 2015, and global processed meat market is anticipated to rise from USD 450.2 billion in 2016 to USD 874.45 by 2021. Keeping the above facts in mind about the rising demand of the fermented foods, novel innovations are quite important for food and beverage industries toward catering the need. The prospective benefits of consuming fermented foods can be ensured only in a safe and contamination-free environment to prevent risk of disease, illness, and death. Innovations should focus on necessary steps to eliminate such possibilities. Further, recombinant DNA technology has provided new lights to the quality and overproduction of desirable products and suppressing the unwanted metabolite. Hence, the future direction of research of food scientists and entrepreneur should focus on bulk production and silencing of undesirable genes through genetic manipulation; simultaneously, it should pass through proper testing to ensure no or least possible damage to human health, environment, and ecology.

References

Adebiyi JA, Obadina AO, Adebo OA, Kayitesi E (2017) Comparison of nutritional quality and sensory acceptability of biscuits obtained from native, fermented, and malted pearl millet (*Pennisetum glaucum*) flour. Food Chem 232:210–217

Adebo OA, Njobeh PB, Adebiyi JA, Gbashi S, Phoku JZ, Kayitesi E (2017a) Fermented pulse-based foods in developing nations as sources of functional foods. In: Hueda MC (ed) Functional food–improve health through adequate food. InTech, Croatia. Accepted, DOI: 10.5772/intechopen.69170. Available from: https://www.intechopen.com/books/functional-food-improve-health-through-adequate-food/fermented-pulse-based-food-products-in-developing-nations-as-functional-foods-and-ingredients

Adebo OA, Njobeh PB, Mulaba-Bafubiandi AF, Adebiyi JA, Desobgo ZSC, Kayitesi E (2017b) Optimization of fermentation conditions for ting production using response surface methodology. J Food Process Preserv 42(1):e13381. https://doi.org/10.1111/jfpp.13381

Adewumi GA, Oguntoyinbo FA, Keisam S, Romi W, Jeyaram K (2013) Combination of culture-independent and culture-dependent molecular methods for the determination of bacterial community of iru, a fermented *Parkia biglobosa* seeds. Front Microbiol. https://doi.org/10.3389/fmicb.2012.00436

Ahren IL, Xu J, Onning G, Olsson C, Ahrne S, Molin G (2014) Antihypertensive activity of blueberries fermented by *Lactobacillus plantarum* DSM 15313 and effects on the gut microbiota in healthy rats. Clin Nutr 34:719–726

Ali R, Athar M, Abdullah U, Abidi SA, Qayyum M (2009) Nutraceuticals as natural healers: emerging evidences. Afr J Biotechnol 8(6):891–898

Aliakbarian B, Palmieri D, Casazza AA, Palombo D, Perego P (2012) Antioxidant activity and biological evaluation of olive pomace extract. Nat Prod Res 26(24):2280–2290

Aliakbarian B, Casale M, Paini M, Casazza AA, Lanteri S, Lanteri S (2015) Production of a novel fermented milk fortified with natural antioxidants and its analysis by NIR spectroscopy. LWT Food Sci Technol 62:376–383

Alkema W, Boekhorst J, Wels M, van Hijum SA (2016) Microbial bioinformatics for food safety and production. Brief Bioinform 17(2):283–292

Barbosa-Canovas GV, Pierson MD, Zhang QH, Schaffner DW (2001) Pulsed electric fields. J Food Sci s8:65–79

Bartra E, Casado M, Carro D, Campama C, Pina B (2010) Differential expression of thiamine biosynthetic genes in yeast strains with high and low production of hydrogen sulfide during wine fermentation. J Appl Microbiol 109:272–281

Biesalski HK (2005) Meat as a component of a healthy diet-are there any risks or benefits if meat is avoided in the diet? Meat Sci 70(3):509–524

Bokulich NA, Mills DA (2012) Next-generation approaches to the microbial ecology of food fermentations. BMB Rep 45:377–389

Brookes G, Barfoot P (2014) Economic impact of GM crops: the global income and production effects 1996–2012. GM Crops Food 5(1):65–75

Caplice E, Fitzgerald GF (1999) Food fermentations: role of microorganisms in food production and preservation. Int J Food Microbiol 50:131–149

Capozzi V, Russo P, Duenas MT, Lopez P, Spano G (2012) Lactic acid bacteria producing B-group vitamins: a great potential for functional cereals products. Appl Microbiol Biotechnol 96:1383–1394

Comi G, Manzano M (2008) Beer production. In: Cocolin L, Ercolini D (eds) Molecular techniques in the microbial ecology of fermented foods. Springer International Publishing, Switzerland, pp 193–207

Csutak O, Sarbu I (2018) Genetically modified microorganisms. In: Holban AM, Grumezescu A (eds) Genetically engineered foods. Academic Press, Cambridge, pp 143–175

Dahabieh MS, Husnik JI, van Vuuren HJH (2009) Functional expression of the DUR3 gene in a wine yeast strain to minimize ethyl carbamate in chardonnay wine. Am J Enol Vitic 60:537–541 da Silva VM, Minim VPR, Ferreira MAM, Souza PHP, Moraes LES, Minim LA (2014) Study of the perception of consumers in relation to different ice cream concepts. Food Qual Pref 36:161–168

de Vrese M, Schrezenmeir J (2008) Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol 111:1–66

Di Cagno R, Minervini G, Rizzello CG, De Angelis M, Gobbetti M (2011) Effect of lactic acid fermentation on antioxidant, texture, color and sensory properties of red and green smoothies. Food Microbiol 28:1062–1071

Dickerson R, Banerjee J, Rauckhorst A, Pfeiffer DR, Gordillo GM, Khanna S, Osei K, Roy S (2015) Does oral supplementation of a fermented papaya preparation correct respiratory burst function of innate immune cells in type 2 diabetes mellitus patients? Antioxid Redox Signal 22:339–345

Doder R, Vukic V, Hrnjez D, Milanovic S, Ilicic M (2013) Health benefits of probiotics application. Food Indust-Milk Dairy Prod 24:3–7

FAO (2016) http://www.faoorg/docrep/005/y2772e/y2772e04htm

FAO/WHO (2001) Report of a Joint FAO/WHO Expert Consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live LAB. Food and Agriculture Organization of the United Nations World Health Organization

Ferrante MA (1996) Keeping it flexible. Food Eng 68(9):143–150

Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66:365–378

Gupta S, Abu-Ghannam N (2012) Recent advances in the application of non thermal methods for the prevention of salmonella in foods. In: Mahmoud BSM (ed) Salmonella – a dangerous food borne pathogen. Intech, Croatia. http://www.intechopen.com/books/salmonella-a-dangerous-foodborne-pathogen/recent-advances-in-theapplication-of-non-thermal-methods-for-the-prevention-of-salmonella-in-foods

Halah MF, Nayra SM (2011) Use of natural plant antioxidant and probiotic in the production of novel yogurt. J Evol Biol 3(2):12–18

Han X, Yang Z, Jing X, Yu P, Zhang Y, Yi H, Zhang L (2016) Improvement of the texture of yogurt by use of exopolysaccharide producing lactic acid bacteria. BioMed Res Int. https://doi.org/10.1155/2016/7945675

Holck A, Heir E, Johannessen T, Axelsson L (2015) North European products. In: Toldra F (ed) Handbook of fermented meat and poultry, 2nd edn. Wiley Blackwell, West Sussex, pp 313–320

https://www.bkwine.com/ (n.d.) Accessed on 10.09.2017

http://www.grandviewresearch.com/press-release/global-functional-foods-market (n.d.) Accessed on 10.09.2017

http://www.worldatlas.com/articles/top-10-beer-producing-nations.html (n.d.) Accessed on 10.09.2017

Hufner E, Britton RA, Roos S, Jonsson H, Hertel C (2008) Global transcriptional response of *Lactobacillus reuteri* to the sourdough environment. Syst Appl Microbiol 31:323–338

Humblot C, Guyot JP (2009) Pyrosequencing of tagged 16SrRNA gene amplicons for rapid deciphering of the microbiomes of fermented foods such as pearly millet slurries. Appl Environ Microbiol 75:4354–4361

Husnik JI, Delaquis PJ, Cliff MA, van Vuuren HJJ (2007) Functional analyses of the malolactic wine yeast ML01. Am J Enol Vitic 58:42–52

Jagani H, Hebbar K, Gang SS, Raj PV, Chandrashekhar HR, Rao JV (2010) An overview of fermenter and the design considerations to enhance its productivity. Pharmacologyon-line 1:261–301

James C (2013) Global status of commercialized biotech/GM Crops: 2013, ISAAA Brief No. 46, Ithaca, NY. http://www.isaaa.org/resources/publications/briefs/49/executivesummary/pdf/b49-execsum-english.pdf. Accessed 04.09.16

Kamiya S, Owasawara M, Arakawa M, Hagimori M (2013) The effect of lactic acid bacteria-fermented soybean milk products on carrageenan-induced tail thrombosis in rats. Biosci Microbiota Food Health 32:101–105

Kaur S, Das M (2011) Functional foods: an overview. Food Sci Biotechnol 20(4):861–875

Kellershohn J, Russell I (2015) Innovations in alcoholic beverage production. In: Ravindra P (ed) Advances in bioprocess technology. Springer International Publishing, Switzerland, pp 423–433

Khan RS, Grigor JV, Win AG, Boland M (2014) Differentiating aspects of product innovation processes in the food industry. Brit Food J 116(8):346–1368

Kim J, Alizadeh P, Harding T, Hefner-Gravink A, Klionsky DJ (1996) Disruption of the yeast *ATH1* gene confers better survival after dehydration, freezing, and ethanol shock: potential commercial applications. Appl Environ Microbiol 62(5):1563–1569

Klaenhammer TR, Azcarate-Peril MA, Altermann E, Barrangou R (2007) Influence of the dairy environment on gene expression and substrate utilization in lactic acid bacteria. J Nutr 137:748S–750S

Korhonen H (2009) Milk-derived bioactive peptides: from science to applications. J Funct Food 1(2):177–187

Krogerus K, Magalhaes F, Vidgren V, Gibson B (2015) New lager yeast strains generated by interspecific hybridization. J Ind Microbiol Biotechnol 42:769–778

Kumar KK, Swain MR, Panda SH, Sahoo UC, Ray RC (2008) Fermentation of litchi (*Litchi chinensis* Sonn.) fruits into wine. Food Rev 2:43–47

Kuswandi B, Wicaksono Y, Abdullah A, Heng LY, Ahmad M (2011) Smart packaging: sensors for monitoring of food quality and safety. Sens & Instrumen Food Qual 5(3–4):137–146

Kwon DY, Daily JW, Kim HJ, Park S (2010) Antidiabetic effects of fermented soybean products on type 2 diabetes. Nutr Res 30:1–13

Law BA, Hansen EB (1997) Classification and identification of bacteria important in the manufacture of cheese. In: Law BA (ed) Microbiology and biochemistry of cheese and fermented milk. Springer, Boston, pp 50–56

Lee JE, Lee BJ, Chung JO, Shin HJ, Lee SJ, Lee CH, Hong YS (2011) ^{1}H NMR-based metabolomic characterization during green tea (*Camellia sinensis*) fermentation. Food Res Int 44:597–604

Leite AM, Mayo B, Rachid CT, Peixoto RS, Silva JT, Paschoalin VM, Delgado S (2012) Assessment of the microbial diversity of Brazilian kefir grains by PCR-DGGE and pyrosequencing analysis. Food Microbiol 31(2):215–221

Lopetcharat K, Choi YJ, Park JW, Daeschel MA (2001) Fish sauce products and manufacturing: a review. Food Rev Int 17(1):65–88

Ma Y, Liu J, Hou J et al (2014) Oral administration of recombinant *Lactococcus lactis* expressing HSP65 and tandemly repeated P277 reduces the incidence of type I diabetes in non-obese diabetic mice. PLoS One 9(8):e105701

Marsh AJ, Hill C, Ross RP, Cotter PD (2014) Fermented beverages with health promoting potential: past and future perspectives. Trend Food Sci Technol 38:113–124

Melikoglu M (2012) Solid-state fermentation of wheat pieces by *Aspergillus oryzae*: effects of microwave pretreatment on enzyme production in a biorefinery. Int J Green Energy 9:529–539

Mirza-Aghayan M, Zonoubi S, Tavana MM, Boukherroub R (2015) Ultrasound assisted direct oxidative esterification of aldehydes and alcohols using graphite oxide and oxone. Ultrason Sonochem 22:359–364

Mishra SS, Ray RC, Panda SK, Montet D (2017) Technological innovations in processing of fermented foods. In: Ray RC, Montet D (eds) Fermented food part II: technological interventions. CRC Press, Boca Raton, pp 21–45

Montoro BP, Benomar N, Lerma LL et al (2016) Fermented Alorena table olives as a source of potential probiotic *Lactobacillus pentosus* strains. Front Microbiol 7. https://doi.org/10.3389/fmicb.2016.01583

Neffe-Skocinska K, Okon A, Kolozyn-Krajewska et al (2017) Amino acid profile and sensory characteristics of dry fermented pork loins produced with a mixture of probiotic starter cultures. J Sci Food Agric 97:2953–2960

Nonaka Y, Izumo T, Izumi F, Maekawa T, Shibata H, Nakano A, Kishi A, Akatani K, Kiso Y (2008) Antiallergic effects of *Lactobacillus pentosus* strain S-PT84 mediated by modulation of Th1/Th2 immunobalance and induction of IL-10 production. Int Arch Allergy Immunol 145:249–257

Nyiri LK (1972) Application of computers in biochemical engineering. In: Squires R (ed) Advances in biochemical engineering, vol 2. Springer, Berlin, pp 49–95

Oey I, Lille M, Van Loey A, Hendrickx M (2008) Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. Trends Food Sci Technol 19:320–328

Oh MR, Park SH, Kim SY, Back HI, Kim MG, Jeon JY, Ha KC, Na WT, Cha YS, Park BH, Park TS, Chae SW (2014) Postprandial glucose-lowering effects of fermented red ginseng in subjects with impaired fasting glucose or type 2 diabetes: a randomized, double-blind, placebo-controlled clinical trial. BMC Comp Alt Med 14. https://doi.org/10.1186/1472-6882-14-237

Panda SK, Ray RC (2016) Fermented foods and beverages from roots and tubers. In: Sharma HK, Njintang NY, Singhal RS, Kaushal P (eds) Tropical roots and tubers: production, processing and technology, 1st edn. Wiley, West Sussex, pp 225–252

Panda SK, Sahu UC, Behera SK, Ray RC (2014a) Bio-processing of bael [*Aegle marmelos* L.] fruits into wine with antioxidants. Food Biosci 5:34–41

Panda SK, Sahu UC, Behera SK, Ray RC (2014b) Fermentation of sapota (*Achras sapota* Linn.) fruits to functional wine. Forum Nutr 13(4):179–186

Panda SK, Panda SH, Swain MR, Ray RC, Kayitesi E (2015) Anthocyanin rich sweet potato (*Ipomoea batatas* L.) beer: technology, biochemical and sensory evaluation. J Food Process Preserv 39(6):3040–3049

Panda SK, Behera SK, Kayitesi E, Mulaba-Bafubiandi AF, Sahu UC, Ray RC (2016) Bioprocessing of jackfruit (*Artocarpus heterophyllus* L.) pulp into wine: technology, proximate composition and sensory evaluation. Afr J Sci Technol Innov Dev 8(1):27–32

Panda SK, Behera SK, Qaku XW, Sekar S, Ndinteh DT, Nanjundaswamy HM, Ray RC, Kayitesi E (2017) Quality enhancement of prickly pears (Opuntia sp.) juice through probiotic fermentation using *Lactobacillus fermentum* ATCC 9338. LWT Food Sci Technol 75:453–459

Park K-Y, Jeong J-K (2016) Kimchi (Korean fermented vegetables) as a probiotic food. In: Watson RR, Preedy VR (eds) Probiotic, prebiotics and synbiotics. Bioactive foods in health promotion. Academic Press, London, pp 391–408

Penacho V, Valero E, Gonzalez R (2012) Transcription profiling of sparkling wine second fermentation. Int J Food Microbiol 153:176–182

Rastall RA, Gibson GR (2015) Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. Curr Opin Biotechnol 32:42–46

Realini CE, Marcos B (2014) Active and intelligent packaging systems for a modern society. Meat Sci 98(3):404–419

Reddy LV, Reddy OV (2005) Production and characterization of wine from mango fruit (*Mangifera indica* L). World J Microbiol Biotechnol 21(8–9):1345–1350

Rodgers S (2008) Novel applications of live bacteria in food services: probiotics and protective cultures. Trend Food Sci Tech 19:188–197

Rodriguez H, Curiel JA, Landete JM, de las Rivas B, Lopez de Felipe F, Gomez-Cordoves C, Mancheno JM, Muñoz R (2009) Food phenolics and lactic acid bacteria. Int J Food Microbiol 132:79–90

Ronteltap A, van Trijp JCM, Renes RJ, Frewer LJ (2007) Consumer acceptance of technology-based food innovations: lessons for the future of nutrigenomics. Appetite 49:1–17

Rossignol T, Postaire O, Storaï J, Blondin B (2006) Analysis of the genomic response of a wine yeast to rehydration and inoculation. Appl Microbiol Biotechnol 71:699–712

Saad N, Delattre C, Urdaci M, Schmitter JM, Bressollier P (2013) An overview of the last advances in probiotic and prebiotic field. LWT Food Sci Technol 50:1–16

Sahu UC, Panda SK, Mohapatra UB, Ray RC (2012) Preparation and evaluation of wine from tendu (*Diospyros melanoxylon* L) fruits with antioxidants. Int J Food Ferment Technol 2(2):171–178

Samaranayaka AGP, Li-Chan ECY (2011) Food-derived peptidic antioxidants: a review of their production, assessment, and potential applications. J Funct Food 3(4):229–254

Santos CC, Libeck BD, Schwan RF (2014) Co-culture fermentation of peanut-soy milk for the development of a novel functional beverage. Int J Food Microbiol 186:32–41

Sheehan VM, Ross P, Fitzgerald GF (2007) Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. Innov Food Sci Emerg Technol 8:279–284

Sheih IC, Fang TJ, Wu TK, Chen RY (2014) Effects of fermentation on antioxidant properties and phytochemical composition of soy germ. J Sci Food Agric 94:3163–3170

Shima J, Kuwazaki S, Tanaka F, Watanabe H, Yamamoto H, Nakajima R, Tokashiki T, Tamura H (2005) Identification of genes whose expressions are enhanced or reduced in baker's yeast during fed-batch culture process using molasses medium by DNA microarray analysis. Int J Food Microbiol 102(1):63–71

Shori AB, Baba AS (2013) Antioxidant activity and inhibition of key enzymes linked to type-2 diabetes and hypertension by *Azadirachta indica*-yogurt. J Saudi Chem Soc 17(3):295–301

Siefarth C, Bich T, Tran T, Mittermaier P, Pfeiffer T, Buettner A (2014) Effect of radio frequency heating on yoghurt, I: technological applicability, shelf-life and sensorial quality. Food Rev 3:318–335

Solieri L, Dakal TC, Giudici P (2013) Next-generation sequencing and its potential impact on food microbial genomics. Ann Microbiol 63:21–37

Stewart GG (2016) *Saccharomyces* species in the production of beer. Beverages 2(4):34. https://doi.org/10.3390/beverages2040034

Swain MR, Anandharaj M, Ray RC, Parveen RR (2014) Fermented fruits and vegetables of Asia: a potential source of probiotics. Biotechnol Res Int. https://doi.org/10.1155/2014/250424

Tai SL, Daran-Lapujade P, Walsh MC, Pronk JT, Daran J (2007) Acclimation of *Saccharomyces cerevisiae* to low temperature: a chemostat-based transcriptome analysis. Mol Biol Cell 18:5100–5112

Tan J, Dai W, Lu M, Lv H, Guo L, Zhang Y, Zhu Y, Peng Q, Lin Z (2016) Study of the dynamic changes in the non-volatile chemical constituents of black tea during fermentation processing by a non-targeted metabolomics approach. Food Res Int 79:106–113

Tao Y, Garcia J, Sun D-W (2014) Advances in wine aging technologies for enhancing wine quality and accelerating wine aging process. Crit Rev Food Sci Nutr 54:817–835

Todorov SD, Holzapfel WH (2015) Traditional cereal fermented foods as sources of functional microorganisms. In: Holzapfel WH (ed) Advances in fermented foods and beverages: improving quality, technologies and health benefits. Elsevier, UK, pp 123–153

Udenigwe CC, Mohan A (2014) Mechanisms of food protein-derived antihypertensive pep- tides other than ACE inhibition. J Funct Food 8:45–52

USFDA (2007). US Food and Drug Administration, 2007. http://www.fda.gov/Food/FoodIngredientsPackaging/IrradiatedFoodPackaging/ucm110564.htm#authors

Varankovich NV, Nickerson MT, Korber DR (2015) Probiotic-based strategies for therapeutic and prophylactic use against multiple gastrointestinal diseases. Front Microbiol 6:685

Verstrepen KJ, Chambers PJ, Pretorius IS (2006) The development of superior yeast strains for the food and beverage industries: challenges, opportunities, and potential benefits. In: Querol A, Fleet G (eds) The yeast handbook: yeasts in food and beverages, vol 2. Springer-Verlag, Berlin, pp 399–444

Vitali B, Minervini G, Rizzello CG, Spisni E, Maccaferri S, Brigidi P et al (2012) Novel probiotic candidates for humans isolated from raw fruits and vegetables. Food Microbiol 31(1):116–125

Volzing K, Borrero J, Sadowsky MJ et al (2013) Antimicrobial peptides targeting gram-negative pathogens, produced and delivered by lactic acid bacteria. ACS Synth Biol 2:643–650

Wakil SM, Laba SA, Fasika SA (2014) Isolation and identification of antimicrobial-producing lactic acid bacteria from fermented cucumber. Afr J Biotechnol 13(25):2556–2564

Walker GM, Hill AE (2016) *Saccharomyces cerevisiae* in the production of whisk(e)y. Beverages 2(4):38. https://doi.org/10.3390/beverages2040038

Wang J-J, Wang Z-Y, Liu X-F, Guo X-N, He X-P, Wensel PC et al (2010) Construction of an industrial brewing yeast strain to manufacture beer with low caloric content and improved flavor. J Microbiol Biotechnol 20(4):767–774

Wegkamp A, Starrenburg M, de Vos WM, Hugenholtz J, Sybesma W (2003) Transformation of the folate-consuming *Lactobacillus gasseri* into a folate-producer. Appl Environ Microbiol 70(5):3146–3148

Wei C, Xun AY, Wei XX et al (2016) Bifidobacteria expressing tumstatin protein for antitumor therapy in tumor-bearing mice. Technol Cancer Res Treat 15:498–508

Wong AY, Chan AW (2016) Genetically modified foods in China and the United States: a primer of regulation and intellectual property protection. Food Sci Hum Wellness 5(3):124–140

Wyrwa J, Barska A (2017) Innovations in the food packaging market: active packaging. Eur Food Res Technol 243:1681–1692

Xu L, Du B, Xu B (2015) A systematic, comparative study on the beneficial health components and antioxidant activities of commercially fermented soy products marketed in China. Food Chem 174:202–213

Yeo SK, Liong MT (2010) Angiotensin I-converting enzyme inhibitory activity and bioconversion of isoflavones by probiotics in soymilk supplemented with prebiotics. Int J Food Sci Nutr 61:161–181

Yerlikaya O (2014) Starter cultures used in probiotic dairy product preparation and popular probiotic dairy drinks. Food Sci Tech (Campinas) 34(2):221–229

Zhang C, Wohlhueter R, Zhang H (2016) Genetically modified foods: a critical review of their promise and problems. Food Sci Hum Wellness 5(3):116–123

Chapter 2
Lactic Acid Bacteria and Yeasts as Starter Cultures for Fermented Foods and Their Role in Commercialization of Fermented Foods

Sujatha Kandasamy, Digambar Kavitake, and Prathapkumar Halady Shetty

Abstract Consumption of fermented foods has substantially increased in the recent years due to their valuable traits that extend well beyond shelf life, preservation and sensory qualities. These foods turn out to play a central role in the diet of several cultures because of its enriched health benefits that are known to possess antimicrobial, antidiabetic, anti-atherosclerotic, antioxidant and anti-inflammatory activities. Consequently, fermentable microorganisms, fermentation process and its products draw scientific interest. Currently fermented food production is mainly carried out using starter cultures for a precise and expectable fermentation. Lactic acid bacteria (LAB) and yeast are the highly studied starters applied in several fermented food production industries such as dairy, meat, sourdough, vegetables, etc. Advanced genetic approaches towards selection of promising organisms can meet the huge demand in starter culture markets along with providing functional value to some traditional food products. This chapter outlines about fermented foods, starter culture types, selection criteria, starter culture markets, role and application of LAB and yeast in fermented foods.

Keywords Starter culture · Fermented foods · Lactic acid bacteria · Yeast

Introduction

Fermented foods occupy a central position in our diet and are widely consumed for ages. Preparation of fermented foods under household or in small-scale units involves fairly simple practices and equipment. Fermentation is an older and cost-effective technique developed for producing and preserving foods from food

S. Kandasamy · D. Kavitake · P. H. Shetty (✉)
Department of Food Science and Technology, Pondicherry University, Pondicherry, India

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_2

spoilage (Hutkins 2006). Besides preservation, enrichment of flavour, improved digestibility and enhancement of nutritive and medicinal values are additional advantages (Ebner et al. 2014; Chilton et al. 2015). By the mid-nineteenth century, industrialization of fermented foods and discovery of microorganisms had a significant impact on expanding the fermented products as well as fermentation processes using well-defined cultures. Interestingly, yeast and lactic acid bacteria (LAB) are the main players contributing towards major fermentation processes in numerous commercial-related processes that include milk, bread, vegetables and meat (Katina and Poutanen 2013; Faria-Oliveira et al. 2015). Although there is an elevation in fermented food production through large-scale and sophisticated technologies, still in some countries, traditional fermentation is practised due to its superior flavour and aroma traits. However, it seems to be unavoidable and even strange when such foods become more prevalent and their demand rises; the simple approach to expand the market can be met by upscaling the industrial process in which use of starter cultures is a key factor (Caplice and Fitzgerald 1999; Erten et al. 2014).

Currently starter cultures for fermented foods are improved by strategy instead of screening. The standards of strategy are grounded based on understanding the metabolism and physiology of the organisms along with their significance in food products. Advances in genomics will provide us abundant data towards strategy development on a sound basis much easier. Genomics, proteomics and metabolomics along with laboratory automation and high-throughput selection techniques comprise the food-grade tools for designing (Hansen 2002; Geis 2003). Evolution of new data will significantly improve the upcoming regulation patterns. It is really tough to predict about its impact on the prospects of regulatory needs in novelty of the food industry at the present moment. Biotechnological applications can be an upholding force in producing superior and benign products or restricting the usage of few strains as starter cultures with certified authorization. Recent developments led to establishment of starter cultures in the fields of biosafety, probiotics, enhancement of yield and function of the prevailing culture market and possible application of cultures in other fermentable products (Cogan et al. 2007).

Fermented Foods

Fermentation is a biotechnological process (Fig. 2.1) brought out by microorganisms (bacteria, fungi, yeast, or a combination of them) in anaerobic conditions used by man since, dating back at least 6000 years (McGovern et al. 2004). During the process, fermentable carbohydrates are converted into end metabolites such as alcohols, organic acids and carbon dioxide. The foremost goal for its function was primarily on food preservation and to extend the shelf life, while safety, nutritional value and organoleptic quality of the foods are concurrently succeeded (Sicard and Legras 2011).

Numerous lesser identified fermented foods are used as traditional foods in all over the world. Still now, fermented foods are extensively consumed by both low- and high-income people. Each community in the world follows its own beliefs in fermented foods that signify the tradition, ritual and agro-economic and

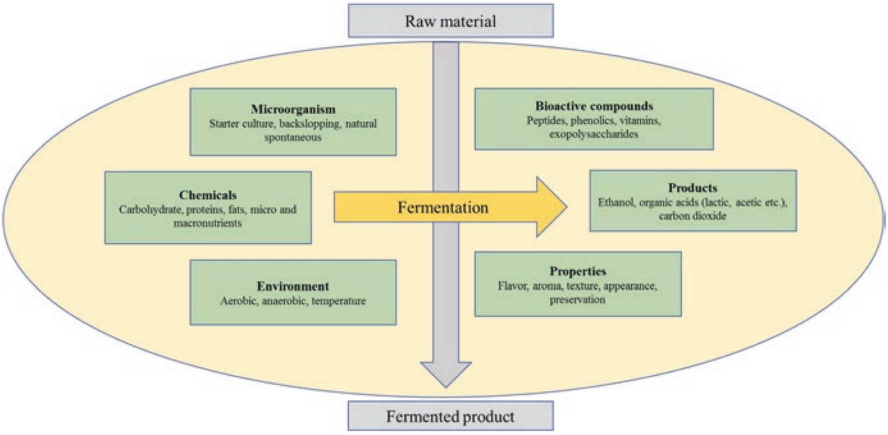


Fig. 2.1 Overview of the role of fermentation mechanism

sociocultural traits of their society. In India, South, East, North-East and Himalayan regions are regarded as hot spot for fermented foods (Ray et al. 2016). Some fermented foods are prevalent as delightful regular dish and boosted globally for its functional, nutritional and medicinal values, yet owing to modern civilization in Asia and Africa, there is a remarkable shift from traditional food habits to fast foods with high calorific value that decline the practicality of traditional fermented foods (Tamang et al. 2016). Several reviews were published on chemical, nutritional and biological components of fermented foods from countries such as Asia (Steinkraus 2002; Rhee et al. 2011; Tamang et al. 2016); Africa (Chelule et al. 2010; Oguntoyinbo et al. 2011; Benkerroum 2013) and America (Nout 2003).

Fermented foods have a huge rising international market and are stared as solitary of the most dynamically explored zone in food science. The progress in fame of fermented foods is ascribed towards consumer well-being, increasing health care and awareness in the numerous benefits of functional foods that lead them for their production at an industrial level. Widespread advantages of fermented foods comprise disease resistance, healthiness and deterrence of nutrition-related diseases. Currently, the significance of fermented foods for consumers is emphasized by the comprehensive variety of fermented products promoted in both industrialized and developing countries, not only for their assured benefit of safety and preservation but also for its highly acceptable sensory traits aided by microorganisms and their enzymes (Selhub et al. 2014; Marco et al. 2017).

Types of Fermented Foods

Overall fermented foods are categorized (Fig. 2.2) into nine categories based on substrates utilized from plant/animal origin: (1) fermented milk products, (2) fermented meat products (3) fermented cereals, (4) fermented legumes,

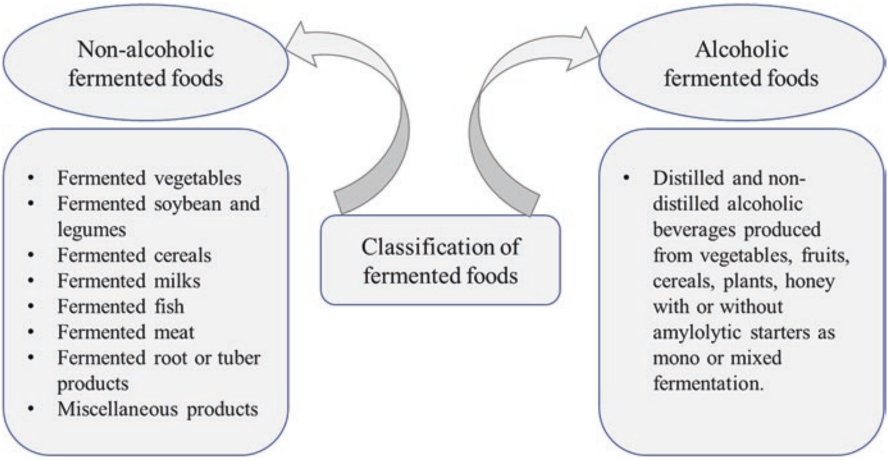


Fig. 2.2 Classification of fermented foods

(5) fermented vegetables and bamboo shoots, (6) fermented roots/tubers, (7) fermented, dried and smoked fish products, (8) miscellaneous fermented products and (9) alcoholic beverages (Tamang et al. 2005; Ray and Sivakumar 2009; Tamang and Kailasapathy 2010).

Health Benefits of Fermented Foods

Fermented foods are known for its promising effect in human health due to its enriched biological compounds synthesized either through oxidation, reduction, hydrolysis, condensation and isomerization (Rai and Jeyaram 2015). Regular intake of fermented foods increases the gut bacterial population up to 10^3-fold making the GI tract more healthy and secure (Kim et al. 2016). In infant formula, these foods stimulate Th1 reactions to balance the immune system as well as to enhance tolerance (Kapsenberg 2003). Kefir, a fermented milk product from kefir grains accumulate remarkable antibacterial (Bourrie et al. 2016), antidiabetic and antiobesity activities (An et al. 2013). Studies on regular intake of yogurt are reported to reduce cardiovascular disease (CVD) (Tapsell 2015) and type 2 diabetes (T2D) (Chen et al. 2014).

Starter Culture

During earlier days, fermentation was applied using inoculum from previous production and used as a starter. A starter culture is a formulation of technological microorganisms that usually consists of a cultivation medium (grains, seeds or nutrient liquids) known to be well inhabited by microorganisms involved in fermentation. With the findings of new microorganisms during the nineteenth century, fermented food production was enhanced utilizing well-characterized starter cultures, thereby accelerating the fermentation activity to derive a wide range of fermented foods with diverse sensorial and dietary characteristics (Holzapfel 2002). Although use of starters in wine, beer, vinegar and bread became a routine from the nineteenth century, dairy and meat industries commenced to use well-characterized starters only after a century later (Hansen 2002). Still spontaneous fermentation has been applied in traditional foods where microflora liable for fermentation are unknown. Mainly, starter cultures are employed for its valuable, safety and technical values which listed elaborately by several institutions such as the International Dairy Federation (IDF), the Food and Drug Administration (FDA), the European Food Safety Authority (EFSA) and the European Food and Feed Cultures Association (EFFCA) (Speranza et al. 2016). Currently, a rationalized inventory on microorganisms (bacteria, moulds or yeasts) used in food fermentations (beverages, cereals, dairy, fish, legumes, meat, vegetables, vinegar) reported 195 species of bacteria and 69 species of yeasts and moulds. Fermentation processes are classified into alcoholic (ethanol production) fermentation predominantly by yeasts and lactic acid fermentation by lactic acid bacteria (LAB) (Wood 2012).

Earlier starter cultures are desired to be formulated just before to use; but nowadays, they are prepared on a commercial scale as freeze-dried material with high concentration of live cultures (Hansen 2002). Choice of starter culture depends upon the substrate or raw material that is involved in fermentation. Starter culture generally comprises bacteria, yeasts, moulds or their combination. Among them, lactic acid bacteria (LAB) and yeasts play a major role in fermentation. Advantageous characteristics of starter cultures involve faster acidification, expectable fermentation activities and desired sensory (aroma, consistency, taste and texture) and safety properties (Holzapfel 2002). Isolation and production of starter cultures were initialized in 1890 from cheese and sour milk in Denmark and Germany. However, recent scientific methods have formulated functional starter cultures, which offer at least one functional trait to enhance the nutrition, quality or safety of the food product.

Selection Criteria

Currently, food industrialization constantly hunts for secure and unique commodities which can be attained via controlled fermentation employing defined starter cultures with specific characteristics. The most appropriate qualities of a starter culture are robustness during production, rapid growth, higher biomass and product yields and typical organoleptic properties (Smid and Kleerebezem 2014). Primarily, starter cultures with precise characteristics were isolated from nature; subsequent in the 1950s, random mutagenesis and selection methods were carried out to expand the usage of starter cultures in fermentation. In this aspect, genetically modified bacteria had lower acceptance, and conventional microbial method is still in practice. However, new DNA sequencing methods facilitate the rapid search for favourable random mutations in the genome. These methods require novel tools for analysing and compiling the obtained data; bioinformatics plays a main role on foreseeing favoured and unfavoured traits of microorganisms, while their growth in diverse food matrices need to be authenticated by laboratory analyses (Sauer 2001).

Although starter cultures are selected based on their suitable safety and 'functional' properties may benefit the consumer, it is more essential to be produced under industrial conditions (Saarela et al. 2000). To develop an ideal culture for any specific food product, it is mandatory to study the function of the culture and to develop methods for improving the function of the culture. Both of the features have advanced substantially via scientific achievements in the last few years. The hunt for a starter culture has till now been depending upon the screening of several isolates in small-scale food fermentations. Selection of starter culture is finalized based on their performance during the process and satisfactory organoleptic analysis of the food product. Tremendous cultures have been isolated using this technique, which in the future will also be used to enlarge the microbial collection to be used as starter cultures. However, in recent years modern tools allow to precisely aim distinct genes and metabolic pathways reliable for vital functioning parameters of a starter culture. Gene targeting allows selection by high-throughput methods feasible, and it initiates the possibility to practise mutant selection and genetic engineering for developing starters that are outstanding to the wild type (Bachmann et al. 2015).

Types of Starter Cultures

In routine, starter cultures can be characterized as mesophilic or thermophilic, depending upon their growth and production temperatures employed for cultivation. Generally, mesophilic cultures raise and yield lactic acid at a mild temperature (30 °C), although thermophilic cultures perform optimally at a higher temperature (42 °C). Examples of mesophilic dairy starters include *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *Leuconostoc mesenteroides* subsp. *cremoris*, whereas thermophilic starters are *Lactobacillus delbrueckii*, *L. helveticus* and *Streptococcus thermophilus* (Cogan et al. 2007).

Nonetheless, general classification of starter cultures depends on culture complexity and the way of reproducing it. Entire starter cultures existing currently are originated in one way or alternative from natural starters of approximate mixture (i.e. comprising an undefined blend of several strains and/or species). For few products, industrial mixed-strain starters (MSS) have replaced the natural starters that have been obtained from the excellent natural starters and cultivated under restricted conditions by particular institutions and industrial starter firms and then supplied to the industries that utilize them for direct vat inoculation or for producing starter in bulk. Because of their long saga, natural starters and commercial MSS are entitled as traditional starters as divergent to defined strain starters (DSS). DSS are generally combined by a small number of individual-specific strains and allow better control on the composition and characteristics of the cultures (De Vuyst 2000).

Traditional starters contain several strains of various microbial types, occasionally involving yeasts and bacteria as well as moulds, which altogether influence biochemically the complexity and the alteration of the end product (Powell et al. 2011). Hence, traditional starter formulation methods are yet in use for few precise or traditional foods and have been amended to a limited commercial scale. Commercial production entails starters that produce reproducible performance and are rid of unwanted organisms. Such targets are problematic to attain under traditional methods. Consequently, DSS have substituted traditional starters in commercial production due to their enhanced, extremely reproducible performance and their high resistance towards phage (Altieri et al. 2016).

Traditional Starters: Natural Starters

The production of natural starters originated from the prehistoric method of backslopping (inoculate a fresh one using inoculum from an earlier fermented item) and/or by subjecting to specific conditions such as low pH, heat or incubation temperature. During starter cultivation, no specific safety measures are required to prevent contamination from the external source, as well as media and culture environments. Due to these consequences, natural starters are constantly developing as approximate blends consisting of numerous cultures and/or species (Carminati et al. 2010). Natural starters are tremendously beneficial pool of strains with suitable technological characteristics such as aroma and antimicrobial production along with phage resistance. Similarly, they seem to be benefited by microbial interactions; moreover, various strains express inadequate acid production capacity when cultured as individual strains (Parente and Cogan 2004).

Mixed-Strain Starters (MSS)/Traditional Starters

MSS, acquired by precise choice of natural starters, are preserved, cultivated and supplied by starter firms and research organizations. Like natural starters, MSS contain an undefined blend of strains that diverge in their physiological and technological characteristics (Parente and Cogan 2004). While undefined strains are grown with few subcultures under controlled environments, the firmness of their composition and function is significantly enhanced with reduced intrinsic variability in contrast to natural strains (Limsowtin et al. 1996). Traditional cultivation of MSS needs numerous shifts to boost the bulk starter by means of minimal quantities of stock cultures that are exchanged using concentrated cultures for inoculation of bulk starter tank, consequently reducing the necessity for shifts inside the factory and the possibility of fluxes in starter components and function (Carminati et al. 2010).

Defined Strain Starters (DSS)

DSS are comprised of one or more cultures (dominant strains of the traditional product) that are preferred, preserved, grown and supplied by specialized companies. Meanwhile, the ratio of strains and/or species in DSS is well-defined; their technological function is highly reproducible which is an advantageous trait. Moreover, currently DSS have substituted traditional starters (Carminati et al. 2010). Conversely, as a concern of the use of few strains, a phage contamination can interrupt the lactic acid fermentation. Additionally, with the consequent failure of natural microbial diversity, conservation of the distinctive features is problematic. However, assessment of the crucial characteristics (growth and acid production, genomic or biochemical traits) of individual species can lead to the rational blend of strains, permissible for culture formulation with appropriate properties (Carminati et al. 2010). DSS are broadly devoid of taste defects and endure a distinguishing feature of 'cleaner' aroma and taste. With the purpose of increasing control over the nature and accomplishing a flavour as similar to the conventional one, industrial firms are manufacturing with intensifying usage of flavour enriching adjunct cultures; DSS strains are included at lower concentration to the starter and can themselves be defined or undefined (Powell et al. 2011).

Production of Industrial Starter Cultures

Starter culture production targets the multiplication of a specific strain or varied population of microorganisms to a concentration that is expected to persist and be metabolically active in the process. Industrial starter culture production is carried in a distinct process, involving suitable quality measures. The selection of starter cultures manufactured on-site in food production or obtained from industrial producers can affect the safety and flexibility of food production. Certain food industries also offer their starter cultures to other firms (Hansen 2002). Usage of freeze-dried or frozen starter cultures excludes the internal reproduction of cultures, thereby reducing the expenses related to bulk culture production, and reduces the possibility of bacteriophage contamination (Santivarangkna et al. 2007).

Production of starter culture was initially started in the early 1800s by Emil Christian Hansen and Christian D.E. Hansen, which has developed into an outstanding international business. Initial starter cultures were in liquid formulation, produced by cultivating bacteria in sterile milk. These cultures deteriorated due to overacidification and hence lost its viability under storage, which might be delayed, through addition of calcium carbonate, but not totally evaded. These problems are overcome by drying method that leads to more stable formulations. Currently, advances in starter culture production stick to accurate procedures with quality standards met by pharmaceutical industry (Taskila 2016).

Conventional processes for production of starter cultures have been defined by Høier et al. (1999) and Buckenhüskes (1993). The foremost phases in industrial production of starter cultures include development and production of a stock culture, culture media preparation, cultivation of stock culture to final cell density in a bioreactor, collection and concentration of cells from medium and culture maintenance. Each single step in the production process of starter cultures is crucial for developing the preferred unique purity and superiority of the culture product. Industrial starter cultures, manufactured by specified companies, are usually supplied in the form of dehydrated or frozen microbial cells. These techniques require culture concentration and dehydration along with preservation to evade loss during storage and distribution (Hansen 2002).

Recent manufacturing practices for industrial starter cultures entail multidisciplinary experience on microbiology and microbial physiology along with process engineering. Moreover, knowledge in cryobiology is necessary when cryotechnologies are engaged for culture preservation (Santivarangkna et al. 2007). Overall quality control comprises numerous steps, such as raw materials analysis, maintenance and control of plant hygiene and evaluation of end products. It is also important to maintain inoculum quality and hygiene, as well as to keep sterile conditions all over the production series. To attain an end product with reliable quality, the conditions for starter culture formulation must be reproducible. Hence, the quality of batches is examined through cell viability and contaminant detection procedures. In general, the industrial production of starter cultures is manufactured by abiding the hazard analysis and critical control point (HACCP) system that also influences the superior quality of fermentation process (Hansen 2002). Starter cultures need to be manufactured in each stage under situations that promote cell survival, preserve cell viability during storage and confirm their accurate functioning in fermentation. Unveiling to stress during various stages of manufacturing may alter the growth and existence of starter cultures. Hence to confirm the appropriate role of cultures, it is crucial to identify and focus on stress-stimulating factors in every stage. Based on the literature, the cultivation phase may expose microbes to starvation, pH changes and metabolite-induced stresses. Oxidative, osmotic, mechanical and thermal stresses are more typical during the later phases of manufacturing, namely, harvesting and preservation, and during storage (Parente and Cogan 2004; Foerst and Santivarangkna 2014).

Role of LAB as Starter Culture

LAB are generally the prominent microorganisms utilized in food industry since the majority of LAB species acquire food-grade status (GRAS, generally recognized as safe). Mostly, LAB ferment the carbon source available in raw foods to lactic acid (Fig. 2.3) with concomitant pH reduction that leads to significant influences like removal of unwanted organisms and enhancement of sensory and texture properties, in addition to impact on health benefits. Further, LAB promote the nutritive value, flavour, tastiness and texture of fermented foods that include dairy products

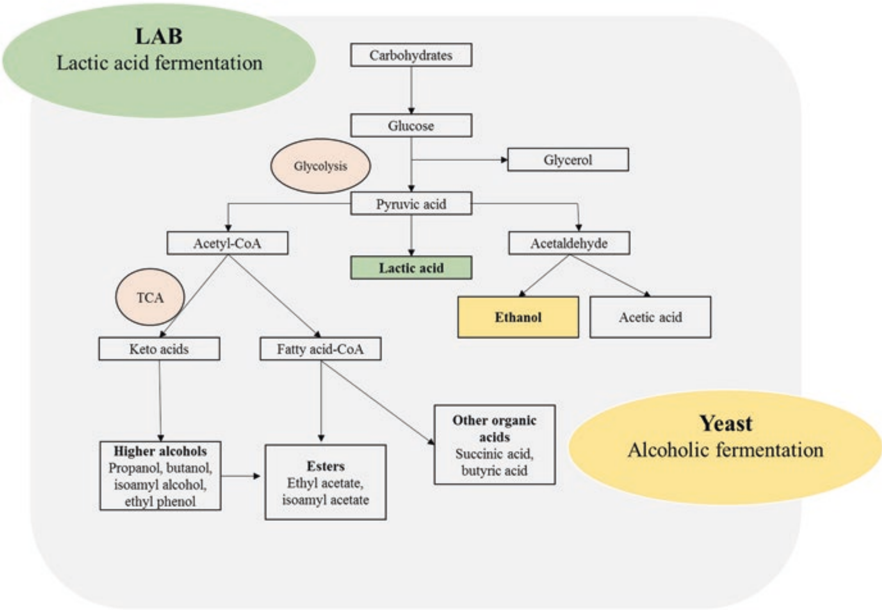


Fig. 2.3 Mechanism of LAB and yeast in fermentation and their products

(buttermilk, cheese, yogurt and fermented milk), fermented beverages, sourdough breads, fermented meats and vegetables (Leroy and De Vuyst 2004; Landete 2017).

LAB are known to produce antimicrobial and aromatic compounds, sugar polymers, vitamins, sweeteners or enzymes that acquire probiotic characteristics. With regard to safety, traits of LAB include source, haemolytic potential, metabolic activities, non-pathogenicity, toxin production, side effects in human experiments and epidemiological surveillance of adverse incidents in consumers (post market). Likewise, functional aspects can be correlated to survival and maintenance in the gastrointestinal (GI) tract, viability under conditions of low and high pH and bile salt tolerance, antibiotic resistance, hydrophobic properties, immunomodulation and antagonistic and antimutagenic properties (Rul et al. 2013; Kleerebezem et al. 2017). Technological aspects include growth at different pH, temperature and salt (NaCl) levels, acidifying ability and metabolism (deamination of arginine, hydrolysis of aesculin, production of acetoin), and they tend to produce adequate flavour/texture (Ruiz-Rodríguez et al. 2017). Regarding the salting effect, supplementation of NaCl is a universal procedure in major fermented dairy foods that also affect the growth of starter culture. Most LAB are partly or wholly retarded at NaCl levels >5%. Though it is clear that salt tolerance is a strain-dependent characteristic, thus this criterion is important in starter selection (Powell et al. 2011).

The foremost function of starter cultures in milk production is peptide degradation to produce small peptides and amino acids by the coagulant. Starter cultures also degrade caseins and convert amino acids to a variety of flavour compounds. The intracellular proteolytic enzymes such as peptidases and amino acid-degrading enzymes are discharged into the cheese matrix through lysis of the cell by the starter cultures for flavour development and also to control bitterness in maturing cheese (Lortal and Chapot-Chartier 2005).

LAB with distinctive traits have been reported from several traditional fermented foods or from various raw materials consumed by starter cultures to produce fermented foods (Table 2.1) (Mohammadi et al. 2012; Montel et al. 2014; Yépez and

Table 2.1 Use of LAB as starter culture in fermented foods

Food products	Raw material	Starter culture	Reference
Kefir	Kefir grains	*Lb. kefir*	Assadi et al. (2000)
		Lb. brevis, *Lb. casei*, *Lb. plantarum*	
		Streptococcus lactis, *Leuc. mesenteroides*	
	Cow's milk	*Lc. lactis*/subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis biovar diacetylactis*, *Leuc. mesenteroides* subsp. *cremoris*, *Lb. plantarum*, *Lb. casei*	Fontán et al. (2006)
Fermented milk	Milk	*Lb. acidophilus*, *Lb. rhamnosus*	Sodini et al. (2002)
		Streptococcus thermophilus	
		Lb. bulgaricus	
		Lb. casei	
		Lb. plantarum	
		Propionibacterium freudenreichii	Baer and Ryba (1992)
		P. jensenii	
Cheddar cheese	Milk	*Streptococcus thermophilus*	Hou et al. (2017)
		Lb. acidophilus, *Lb. casei*, *Lb. paracasei* and *Bifidobacterium* spp.	Ong et al. (2006)
Yoghurt	Sheep milk	*Streptococcus thermophilus*	Michaylova et al. (2007)
		Lb. delbrueckii subsp. *bulgaricus*	
Kimchi	Cabbage	*Weissella cibaria*, *W. confusa* and *W. koreensis*	Lee et al. (2005)
Sauerkraut	Cabbage	*Leuc. mesenteroides*, *Lb. plantarum*, *Lb. casei*	Xiong et al. (2014)
		Lc. lactis	
Sourdough	Wheat	*Lb. brevis*, *Lb. paralimentarius*, *P. pentosaceus*, *W. cibaria*	Paramithiotis et al. (2005)
		Leuc. citreum, *W. koreensis*	Choi et al. (2003)
Fermented olives	Green olives	*Enterococcus casseliflavus*, *Lb. pentosus*,	De Castro et al. (2002)
		Lb. pentosus	Blana et al. (2014)
		Lb. plantarum	
Kivunde	Cassava	*Lb. plantarum*	Kimaryo et al. (2000)
Plaa-som	Fish	*Lb. plantarum*,	Saithong et al. (2010)
		Lb. reuteri	
Sauce	Soybean	*Tetragenococcus*	Singracha et al. (2017)
	Fish	*Halophilus*	Udomsil et al. (2011)
Sausages	Meat	*P. acidilactici*	Leroy et al. (2006)
		P. pentosaceus	
		Lb. pentosus	Coppola et al. (2000)
		Lb. plantarum	
	Pork	*P. pentosaceus*	Kingcha et al. (2012)

Tenea 2015). Numerous LAB species are utilized based on the nature of raw material to produce a desired fermented product. Dairy products like cheese generally contain *Lactococcus* (*Lc.*) *lactis* subsp. lactis, *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis* var. *diacetylactis*, *Leuconostoc* (*Leuc.*) *mesenteroides* subsp. *cremoris*, *Lactobacillus* (*Lb.*) *delbrueckii* subsp. *lactis*, *Lb. helveticus* and *Lb. casei*, the species being dependent on the type of cheese to be produced (Tamime and Thomas 2017). *Leuconostoc* sp. and *L. diacetylactis* are the chief citrate-fermenting LAB found in dairy starters (Drider et al. 2004).

Instead, fermented milks are produced mostly using starters of *Lb. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (yogurt), *Lb. casei*, *Lb. acidophilus*, *Lb. rhamnosus* and *Lb. johnsonii*, whereas other fermented milks (Kefir) comprise *Lb. kefir*, *Lb. kefiranofaciens* and *Lb. brevis* along with yeasts (Leroy and De Vuyst 2004; Mohammadi et al. 2012). Fermented meat products (sausages) involve species such as *Lb. curvatus*, *Lb. sakei*, *Pediococcus (P.) acidilactici* and *P. pentosaceus* (Leroy et al. 2006; Kumar et al. 2017), whereas fermented fish products include *Lb. plantarum*, *Lb. alimentarius and Carnobacterium (C.) piscicola* (Kopermsub and Yunchalard 2010). In fermented vegetables (pickles, sauerkraut and olives), species of *Leuc. mesenteroides*, *Lb. brevis*, *Lb. buchneri*, *Lb. pentosus*, *Lb. plantarum*, *P. acidilactici*, *P. cerevisiae*, *P. pentosaceus*, *Lb. fermentum* and *Tetragenococcus halophilus* are used (Beganović et al. 2014; Elmacı et al. 2015), although in cereal fermentation *Lb. amylovorus*, *Lb. alimentarius*, *Lb. brevis*, *Lb. farciminis*, *Lb. fermentum*, *Lb. plantarum*, *Lb. pontis*, *Lb. panis*, *Lb. reuteri*, *Lb. sanfranciscensis* and *Weissella cibaria* are employed (Katina and Poutanen 2013).

LAB produce several nonspecific antimicrobial substances like short-chain fatty acids, ethanol and hydrogen peroxide, bacteriocins, bacteriocin-like inhibitory substances (BLIS) and antifungal compounds. Due to this functional trait, significance of using LAB has increased as an alternate for the usage of synthetic chemicals and additives in food biopreservation (Dalié et al. 2010). Among the antimicrobial compounds produced by LAB, bacteriocins are the most promising that can be ex situ as food ingredients or in situ using a bacteriocinogenic starter culture (Mokoena 2017).

In food industries, secondary metabolites produced by some starter cultures under in situ are used as flavour compounds in food additives. In cocoa fermentation, *Lb. fermentum* is found to be responsible for the production of relevant flavour compounds (Camu et al. 2007). LAB are capable of producing aroma compounds such as diacetyl, acetaldehyde and esters. Mainly diacetyl-producing LAB include *Lactobacillus* spp., Lc. lactis, *Leuc. mesenteroides* and *Strep. thermophilus* (Hugenholtz et al. 2000), *Lc. lactis biovar diacetylactis* being extensively known for producing large amounts of this compound (Gupta et al. 2015). Acetaldehyde is a notable aroma compound present in yogurt produced using *Lb. delbrueckii* subsp. *bulgaricus* and *Strep. thermophilus* (Cheng 2010). *Lc. lactis* produce ethyl esters and thioesters during cheese production (McSweeney and Sousa 2000; Smit et al. 2005). Numerous LAB strains from goat's and ewe's milk and cheeses reported synthesis of short-chain fatty acid ester (Mukdsi et al. 2013). LAB acts as a potential resource and as an extensive battery for different types of enzymes that modify the components, processing, quality and sensory characteristics of foods and feeds. Several enzymes are produced by LAB into the gastrointestinal tract that put forth possible synergistic effects on digestion and alleviate intestinal malabsorption indications. LAB release enzymes directly into the food matrix while using as a starter or associated cultures or may use as a source of enzymatic extracts to be employed during fermentation conditions (Matthews et al. 2004).

Amylolytic LAB (ALAB) transform starch into lactic acid wholly by combining both saccharification and fermentation in a single step as a cost-effective process (Petrova et al. 2013). ALAB strains might be functional during fermentation of cereal-based foods and beverages (Blandino et al. 2003) in addition to probiotics or hypoallergenic children's foods (Nguyen et al. 2007; Petrova and Petrov 2011). ALAB-producing extracellular amylases are used as starters in sourdough technology for improving bread texture and shelf life of the product (Reddy et al. 2008). Proteases and peptidases from LAB have been utilized in cheese ripening processes to obtain varied sensorial characteristics during glycolysis, lipolysis and proteolysis reactions (Reddy et al. 2008). Specific peptidases produced by *Lc. lactis* subsp. *cremoris* enhanced the sensory qualities of cheese (Patel et al. 2013). Alternatively, noncoagulant proteases are used in dairy industry for production of casein and whey hydrolysate (Feijoo-Siota et al. 2014).

Certain gastrointestinal LAB species are able to synthesize B-group vitamins (i.e. folate or vitamin B9, riboflavin or vitamin B2, cobalamin or vitamin B12, menaquinone or vitamin K2). Besides, these vitamins exist in fermented foods by LAB such as cheese, yogurt and cultured buttermilk (LeBlanc et al. 2013). During fermentation, folate content is increased using strains of *Bifidobacterium* along with starters such as *Strep*. *thermophilus* and/or *Lb. delbrueckii* subsp. *bulgaricus* in skim milk, *Lb*. *helveticus* MTCC 5463 and *Lb. rhamnosus* MTCC 5462 in milk and *Strep*. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* in yogurt (Saubade et al. 2016; Rad et al. 2016).

GABA-producing LAB may involve as starters in producing GABA enriched functional foods and beverages, cheese (Park and Oh 2006), dairy products (Hayakawa et al. 2004), black raspberry juice (Kim et al. 2009), kimchi (Seok et al. 2008) and soymilk (Tsai et al. 2006). Moreover, GABA can also be used as a food ingredient; a marketable natural GABA formulation branded as Pharma GABATM produced using *Lb. hilgardii* K-3 is licensed by the US FDA (Takeshima et al. 2014). EPS from LAB have more commercial value because of its immense potential applications in the food industry as a natural and safe food additive for bettering mouthfeel, rheological properties, smoothness, stability, texture and water retention in food products (Welman and Maddox 2003). EPS from *Weissella* spp. and *Lb*. *sanfranciscensis* starters ensured a positive impact on the hardness, stickiness, volume and mouthfeel in cereal and pseudocereal doughs (Korakli et al. 2002; Galle et al. 2010). EPS producing *Lb. mucosae* DPC 6426 are used as starter cultures in yogurt to decrease syneresis and to improve the water retention and rheological characteristics (viscosity and elasticity) (London et al. 2015).

Role of Yeast as Starter Cultures

In olden times people naively used yeasts for producing fermented foods and beverages; the knowledge of these microorganisms' ability to alter carbohydrates into ethanol and carbon dioxide (CO_2) (Fig. 2.3) is demonstrated in the 1860s by Louis Pasteur. Yeasts are eukaryotic unicellular organisms known for its occurrence in a broad range of traditional fermented foodstuffs produced from raw materials of both plant and animal origin (Tamang and Fleet 2009). Yeasts carry a significant function in food fermentation through enzyme production that favours desired biochemical reactions, such as production of flavour, alcohol and aroma (Aidoo et al. 2006). Most of the favourable yeasts for desirable food fermentation belong to the genera *Saccharomyces*, especially *S. cerevisiae*, commonly labelled 'baker's yeast' (Sicard and Legras 2011). Research on further genera of *Candida* spp., *Endomycopsis* spp., *Hansenula* spp., *Pichia* spp., *Rhodotorula* spp., *Saccharomycopsis* spp. and *Torulopsis* spp. is getting attention for their promising utilization as starter cultures in both food and non-food (industrial) applications (Table 2.2) (Buzzini and Vaughan Martini 2006). The main roles of yeast in fermented foods include alcohol production, texture enhancement by leavening, acidification and antitoxin production for preservation, increasing nutritive values and removing anti-nutritional components and producing bioactive peptides and vitamins as value-added products (Romano et al. 2006) (Fig. 2.1).

Yeast is generally used as leavening agents for raise in bakery and pastry products. In dough fermentation process, yeast converts sugar into alcohol and carbon dioxide that govern the textural property of the baking product. *Saccharomyces cerevisiae* or baker's yeast is the most extensively used yeast in bakery product as a leavening agent (Newberry et al. 2002). In addition to gas production, yeasts produce succinic acid known to be responsible for dough rheology and bread flavour (Jayaram et al. 2014). During fermentation of milk, organic acid (acetic acid, butyric acid, lactic acid, propionic acid and pyruvic acid) production is also reported with starter yeasts (Alvarez-Martin et al. 2008). Hence, the choice of a suitable yeast co-starter is mandatory for maintaining limited organic acid production in a required product.

During fermentation, few yeasts in fermented foods are identified to produce hydrolytic enzymes to improve the digestibility of the product and enrich vitamin levels. Hydrolytic (intracellular and extracellular) enzymes such as amylase, cellulase, b-glucosidase, invertase, lipase, pectinase, protease, phytase and xylanase are able to be produced by yeast (Maturano et al. 2012). Yeast are also stated for production of bioactive peptides such as carboxypeptidases and aminopeptidases during hydrolysis of milk proteins (Ferreira and Viljoen 2003).

During carbohydrate fermentation in fermented foods, *S. cerevisiae* produces metabolic products such as carbonyl compounds, esters and organic acids (Jayaram et al. 2013). Yeast supplementation during idli batter fermentation implies the product with acceptable texture and sensory traits (Aidoo et al. 2006). In recent studies, existence of yeast diversity in Kefir worldwide includes genera *Candida*, *Saccharomyces*, *Pichia*, *Kazachatania*, *Kluyveromyces* and *Zygosaccharomyces* (Magalhães et al. 2010; Leite et al. 2012; Garofalo et al. 2015).

Table 2.2 Use of yeast as starter culture in fermented foods

Category	Fermented food	Yeasts starter culture	References
Cereals	Ogi	*Pichia kudriavzevii*	Ogunremi et al. (2015)
	Pozol	*Rhodotorula minuta*, *Rhodotorula mucilaginosa*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Candida guilliermondii*, *Kluyveromyces lactis*	Wacher et al. (2000)
	Sourdough	*Candida humilis*, *Kazachstania exigua*, *Wickerhamomyces anomalus*, *Candida famata*, *Saccharomyces cerevisiae*	Hammes et al. (2005), Vrancken et al. (2010), and Daniel et al. (2011)
	Togwa	*Issatchenkia orientalis*, *Pichia anomala*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus* and *Candida glabrata*	Hjortmo et al. (2008)
Dairy	Kefir	*Kluyveromyces* spp., *Saccharomyces* spp., *Torula* spp., *Williopsis saturnus* var. *saturnus*	Ahmed et al. (2013) and Viljoen (2006)
Vegetables	Cassava	*Candida ethanolica*, *Geotrichum candidum*	Lacerda et al. (2005)
	Pulque	*Candida diversa*, *Kluyveromyces marxianus*, *Pichia fermentans*, *Torulaspora delbrueckii*	Páez-Lerma et al. (2013)
	Kanji	*Rhodotorula glutinis*	Malisorn and Suntornsuk (2008)
Fruits	Chilli pepper	*Hanseniaspora guilliermondii*, *Kodameae ohmeri*, *Rhodotorula* spp., *Debaryomyces* spp., *Cryptococcus* spp.	González-Quijano et al. (2014) and Zhao et al. (2016)
	Olives	*Candida krusei*, *C. boidinii*, *C. parapsilosis*, *C. rugose*, *Pichia anomala*, *P. membranifaciens*, *Debaryomyces hansenii*, *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, *Kluyveromyces marxianus*, *Rhodotorula glutinis*	Coton et al. (2006), Hurtado et al. (2008), and Arroyo-López et al. (2006)
	Cocoa	*Saccharomyces cerevisiae*, *Hanseniaspora opuntiae*	Papalexandratou and De Vuyst (2011)
	Tepache	*Hanseniaspora uvarum*, *Pichia guilliermondii*	Corona-González et al. (2013)

Yeasts are involved in several fermented milk products like Amasi, Kefir, Kumis, Kurut, Laban, Longfil and Viili (Rai and Jeyaram, 2015) for their characteristic typical aroma profile and texture. *Kluyveromyces lactis*, *K. marxianus*, *Debaryomyces hansenii* and *Yarrowia lipolytica* are the commonly reported yeast species in cheese fermentation. Yeast hastens the ripening process and enhances flavour components in numerous cheese products (Alvarez-Martin et al. 2008; Rai and Jeyaram, 2015). During cheese ripening, the lipolytic yeasts produce alcohols, methyl ketones and lactones that perform chief role in flavour production. *Candida zeylanoides* (Fadda et al. 2010), *Debaryomyces hansenii* (Padilla et al. 2014),*Geotrichum candidum* (Tornadijo et al. 1998) and *Trichosporon cutaneum* (Corbo et al. 2001) are the most important yeast species related to be involved in cheese ripening process for flavour improvement. Although varied yeast species have been stated in yogurt fermentation, the most commonly identified is

Saccharomyces cerevisiae. It is remarkable to observe that there is no proof of yeast described from 'Doi' or 'Dahi' (Indian fermented milk), the main fermented food of the locality (De Wit et al. 2005; Rai and Jeyaram, 2015).

Yeasts cause a positive impact on the flavour development in fermented sausages and cured hams (Mauriello et al. 2004; Tamang and Fleet 2009). Several researchers stated an increase in folate content during yeast fermentation in baked products of wheat (Kariluoto et al. 2004) and rye (Katina et al. 2007). In sourdough fermentation, yeast was found to be more effective than LAB in raising the folate content (Kariluoto et al. 2006). Yeast also improves the nutritional quality of bread by increasing the bioavailability and level of sterols, phenols, vitamins and fibre solubilization and boosting the bioavailability of minerals and reduction in starch digestibility (Poutanen et al. 2009).

Yeasts are noted as one of the beneficial microorganisms for producing phytase responsible for degradation of phytic acid, an antinutritional factor (Greppi et al. 2015). During fermentation, degradation of phytate by yeast improves the bioavailability of divalent metals such as calcium, iron, magnesium and zinc in the gastrointestinal tract. The widespread phytase-producing yeast comprises *Saccharomyces cerevisiae*, *S. kluyveri*, *Candida krusei*, *Arxula adeninivorans*, *Debaryomyces castellii*, *Kluyveromyces lactis*, *Pichia anomala*, *P. rhodanensis*, *P. spartinae*, *Rhodotorula gracilis*, *Schwanniomyces castellii* and *Torulaspora delbrueckii* (Moslehi-Jenabian et al. 2010).

In fermented foods, yeast networks with related microbes in a positive way by favouring the starter culture in the final product formation (Viljoen 2001) or by preventing and removing the undesirable microorganisms that deteriorate the product quality (Fleet 2003). Yeast reduces the growth of pathogenic and spoilage organisms in fermented foods by producing antibiotic factors, organic acids, toxins and hydrogen peroxide (Chen et al. 2015). Yeast enhances the lactic acid bacterial population involved in food fermentation by producing vitamins, amino acids and purines and free sugars by conversion of complex carbohydrates that are crucial for anideal lactic acid bacteria growth (Viljoen 2006). Similarly, lactic acid bacteria produce organic acids and reduce the medium pH, thereby establishing an environment that favours the yeast growth (Aidoo et al. 2006).

Yeast do function as a promising probiotic in fermented foods comprising certain yeast species namely *D. hansenii*, *Torulaspora delbrueckii* (Psani and Kotzekidou 2006), *Kluyveromyces lodderae*, *K. marxianus* (Kumura et al. 2004), *K. lactis* and *Yarrowia lipolytica* (Chen et al. 2010) with potent antimicrobial activity beside pathogenic bacteria and the ability to survive in gastrointestinal tract. Application of yeast producing specific health-promoting bioactive metabolites, free polyphenols, peptides and oligosaccharides as a co-starter in fermented food is an additional advantage (Fleet 2003).

Extracellular lipolytic and proteolytic enzymes produced by yeasts improves the sensory characteristics of fermented meats due to release of amino acids, free fatty acids and small peptides during the ripening process (Martín et al. 2006; Patrignani et al. 2007; Andrade et al. 2009). Several current studies in fermented meats have categorized yeast communities in turn to select *D. hansenii*, Candida spp. and *Y. lipolytica* as feasible industrial starters, rarely for mixed fermentation (Sánchez-Molinero and Arnau 2008; Purriños et al. 2013; Kumar et al. 2017).

In olive fermentation, yeasts are reported to produce compounds such as acetaldehyde, esters, ethanol, glycerol, higher alcohols, organic acids and other volatile compounds revealing significant organoleptic traits that improve the quality of fermented olives, mainly by generation of flavour during the process (Montaño et al. 2003; Arroyo López et al. 2012). The lipolytic activity of the yeast is revealed to improve the volatile profile by increasing the free fatty acid content of these foodstuffs. Choice of yeast starters such as *Candida*, *Debaryomyces*, *Kluyveromyces* and *Saccharomyces* in olive fermentation is taken as a crucial step for developing the process in both laboratory and at an industrial scale (Corsetti et al. 2012; Bevilacqua et al. 2013; Pistarino et al. 2013).

In cocoa fermentation, yeast is known to release pulp-degrading pectinases, converting pulp sugars into ethanol and for chocolate aroma development (Ho et al. 2014). *Candida*, *Hanseniaspora*, *Hyphopichia*, *Kodamaea*, *Kluyveromyces*, *Meyerozyma*, *Pichia*, *Saccharomyces*, *Trichosporon* and *Yamadazyma* are the commonly noted genera during cocoa fermentations (Ardhana and Fleet 2003; Boekhout and Samson 2005; Nielsen et al. 2007; Daniel et al. 2009; Papalexandratou and De Vuyst 2011; Lefeber et al. 2012; Crafack et al. 2013).

Pectinolytic yeasts including *S. cerevisiae*, *S. bayanus*, *K. marxianus*, *Pichia kluyveri*, *Schizosaccharomyces* sp. and *Wickerhamomyces anomalus* have been suggested as starters for fermentation of coffee cherries (Jayani et al. 2005; Masoud and Jespersen 2006; Silva et al. 2013). Fermented bamboo shoots, soy sauces, table olive, papad and wadi also engage yeast fermentation. *Zygosaccharomyces rouxii*, salt-tolerant yeast, is highly involved in soy sauce fermentation (Wah et al. 2013).

Starter Culture in the Market

The world market for starter cultures has met encouraging growth in the last few years, mostly owing to commercialization of food production. Arising business prospects are associated to starter culture markets in developing counties of Asia and Africa, where fermented foods play a foremost part in the diet. Defined starters are known to enhance small-scale fermentation processes and their consistency (Sanchez et al. 2001; Coulin et al. 2006), to enrich the aroma in traditional foods (Teniola and Odunfa 2001) and to enhance product safety (Valyasevi and Rolle 2002).

In 2018, international starter culture market price is predicted to be $1.0 billion. Yeast cultures, predominantly consumed during alcoholic beverages production, govern the starter culture market, while bacterial starters utilized mainly in dairy industry occupy the second biggest market. As per stats of 2012, the biggest starter cultures market is Europe, followed by North America. Arising consumer awareness towards health aids regarding probiotics and the ability of end user to hire higher prices for premium products are classic qualities of European markets.

Table 2.3 Commercial starter cultures available in the market

Starter culture company	Product	Country
Alce	Yogurt, cheese, butter	Italy
LB Bulgaricum PLC	Yogurt, white and yellow cheese	Bulgaria
Chr. Hansen	Cheddar, soft, white and cottage cheese, grana, pasta filata	Denmark
Caldwell Bio Fermentation	Fermented vegetables	Canada
CSK food enrichment	Yogurt, cheese, butter	Netherlands
Cultures for health	Yogurt, buttermilk, Kefir, kombucha, cheese, tofu, sour cream, sourdough	USA
Cutting edge cultures	Fermented vegetables, Kefir	USA
Danisco	Cheese, yogurt	Denmark
DSM	Cheese, yogurt	Netherlands
Goldrush	Sourdough	USA
Lactina Ltd.	Cheese, yogurt	Bulgaria
Lesaffre Group	Sourdough, whisky, rum, tequila, mezcal, vodka, cider, sorghum beer, kvass, etc.	France
Lyo-San Yogourmet	Yogurt	USA and Canada
Rhodia	Cheese	France
Wyeast Laboratories Inc.	Beer, cider, wine, wild and sour, mead, sake	USA

Around 2018, the North American market is predicted to an increase of 5.9% compound annual growth rate (CAGR). A rapid increase in starter culture market is observed in the region of Asia Pacific, which is expected to an increase of 6.3% CAGR on the mid-2010s due to promising market and environmental conditions that boost the production of fermented foods. At present, Angel Yeast Co. Ltd. (China), Lallemand Inc. (USA) and Chr. Hansen A/S (Denmark) are the foremost firms of industrial starter cultures. The foremost starter culture industries are mainly dominated by LAB followed by yeast (Table 2.3). Besides few dairy companies like the Finnish company Valio Ltd. market its superior starter cultures to certified clients. Production of starter cultures is also carried out under contracts offered by several companies.

Future Perspectives

Development towards commercialization of food products in worldwide also boosts the evolution of starter culture markets. Arising business opportunities are associated to starter culture markets in developing areas of Asia and Africa, where fermented foods involve foremost role in diet. In developing countries fermented foods play a vital socioeconomic role and meet the protein needs of their populations (Chilton et al. 2015). Traditional fermentation is normally carried out under basic conditions that account to poor functioning process and also low quality and security of the products. Besides, native foods undergo shorter shelf life and normally have less appealing form compared to junk foods that have drastically reformed the food habits in few countries (Achi and Ukwuru 2015).

Traditional foods with superior and balanced sensorial and nutritional quality are the most important factor in improving product standardization in starter activity. There is a huge demand for starter culture due to its wide application in innumerable areas such as beverages, dairy, foods, fruit juice industry, etc. Utilization of commercial starters with limited choice has lessened the biodiversity and organoleptic traits of fermented food products due to restricted availability of novel, fascinating starter cultures. Hence, the choice of favourable wild strains from raw materials might be a fascinating way forward. Increased health awareness among the consumers towards reduction of chemical usage towards antibiotics/preservatives leads to demand in novel starter cultures. To employ as promising starter cultures, characterization of the phenotype and genotype of strains is a prerequisite that comprises both safety and technological characteristics (Ammor and Mayo 2007). Advances in molecular biology techniques via RDNA technology and microarrays, with improved genome sequencing, uncover the opportunities to model and acquire new starters with resistance towards disease and antimicrobial production that focus towards growth of unwanted organisms or alter the prevailing organisms with preferred genes (Capece et al. 2010). Next-generation sequencing extends new perceptions on microbial safety based on whole-genome assessment (Zhang et al. 2012), substantially rising the number of whole genomes of autochthonous strains with functional property (Lamontanara et al. 2014), such as biogenic amine producers (Ladero et al. 2014) and probiotic strains (Treven et al. 2014).

References

Achi OK, Ukwuru M (2015) Cereal-based fermented foods of Africa as functional foods. Int J Microbiol Appl 2(4):71–83

Ahmed Z, Wang Y, Ahmad A, Khan ST, Nisa M, Ahmad H, Afreen A (2013) Kefir and health: a contemporary perspective. Crit Rev Food Sci Nutr 53(5):422–434

Aidoo KE, Nout NJR, Sarkar PK (2006) Occurrence and function of yeasts in Asian indigenous fermented foods. FEMS Yeast Res 6:30–39

Altieri C, Ciuffreda E, Di Maggio B, Sinigaglia M (2016). Lactic acid bacteria as starter cultures. In: Speranza B, Bevilacqua A, Corbo MR, Sinigaglia M. (Eds.). (2016). Starter Cultures in Food Production. John Wiley & Sons., Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, 1–15

Alvarez-Martin P, Florez AB, Hernández-Barranco A, Mayo B (2008) Interaction between dairy yeasts and lactic acid bacteria strains during milk fermentation. Food Control 19(1):62–70

Ammor MS, Mayo B (2007) Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: an update. Meat Sci 76(1):138–146

An SY, Lee MS, Jeon JY, Ha ES, Kim TH, Yoon JY, Han SJ (2013) Beneficial effects of fresh and fermented kimchi in prediabetic individuals. Ann Nutr Metab 63(1–2):111–119

Andrade MJ, Rodríguez M, Casado EM, Bermúdez E, Córdoba JJ (2009) Differentiation of yeasts growing on dry cured Iberian ham by mitochondrial DNA restriction analysis, RAPDPCR and their volatile compounds production. Food Microbiol 26:578–586

Ardhana MM, Fleet GH (2003) The microbial ecology of cocoa bean fermentations in Indonesia. Int J Food Microbiol 86:87–99

Arroyo López FN, Romero Gil V, Bautista Gallego J et al (2012) Potential benefits of the application of yeast starters in table olive processing. Front Microbiol 3:1–4

Arroyo-López FN, Durán-Quintana MC, Ruiz-Barba JL, Querol A, Garrido-Fernández A (2006) Use of molecular methods for the identification of yeast associated with table olives. Food Microbiol 23(8):791–796

Assadi MM, Pourahmad R, Moazami N (2000) Use of isolated kefir starter cultures in kefir production. World J Microbiol Biotechnol 16(6):541–543

Bachmann H, Pronk JT, Kleerebezem M, Teusink B (2015) Evolutionary engineering to enhance starter culture performance in food fermentations. Curr Opin Biotechnol 32:1–7

Baer A, Ryba I (1992) Serological identification of propionibacteria in milk and cheese samples. Int Dairy J 2(5):299–310

Beganović J, Kos B, Pavunc AL, Uroić K, Jokić M, Šušković J (2014) Traditionally produced sauerkraut as source of autochthonous functional starter cultures. Microbiol Res 169(7):623–632

Benkerroum N (2013) Traditional fermented foods of North African countries: technology and food safety challenges with regard to microbiological risks. Compr Rev Food Sci Food Saf 12(1):54–89

Bevilacqua A, Beneduce L, Sinigaglia M, Corbo MR (2013) Selection of yeasts as starter cultures for table olives. J Food Sci 78:742–751

Blana VA, Grounta A, Tassou CC, Nychas GJE, Panagou EZ (2014) Inoculated fermentation of green olives with potential probiotic *Lactobacillus pentosus* and *Lactobacillus plantarum* starter cultures isolated from industrially fermented olives. Food Microbiol 38:208–218

Blandino A, Al-Aseeri ME, Pandiella SS, Cantero D, Webb C (2003) Cereal-based fermented foods and beverages. Food Res Int 36(6):527–543

Boekhout T, Samson R (2005) Fungal biodiversity and food. In: Nout RMJ, de Vos WM, Zwietering MH (eds) Food fermentation. Wageningen Academic, Gelderland, pp 29–41

Bourrie BC, Willing BP, Cotter PD (2016) The microbiota and health promoting characteristics of the fermented beverage kefir. Front Microbiol 7:647

Buckenhüskes HJ (1993) Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. FEMS Microbiol Rev 12:253–272

Buzzini P, Vaughan Martini A (2006) Yeast biodiversity and biotechnology. In: Rosa C, Péter G (eds) The yeast handbook: biodiversity and ecophysiology of yeasts. Springer, Berlin, pp 533–559

Camu N, De Winter T, Verbrugghe K, Cleenwerck I, Vandamme P, Takrama JS, De Vuyst L (2007) Dynamics and biodiversity of populations of lactic acid bacteria and acetic acid bacteria involved in spontaneous heap fermentation of cocoa beans in Ghana. Appl Environ Microbiol 73(6):1809–1824

Capece A, Romaniello R, Siesto G et al (2010) Selection of indigenous *Saccharomyces cerevisiae* strains for Nero d'Avola wine and evaluation of selected starter implantation in pilot fermentation. Int J Food Microbiol 144:187–192

Caplice E, Fitzgerald GF (1999) Food fermentations: role of microorganisms in food production and preservation. Int J Food Microbiol 50(1):131–149

Carminati D, Giraffa G, Quiberoni A, Binetti A, Suarez V, Reinhemer J (2010) Advances and trends in starter culture for dairy fermentation. In: Mozzi F, Raya RR, Vignolo GM (eds) Biotechnology of lactic acid bacteria: novel applications. Blackwell, Oxford, pp 177–192

Chelule PK, Mbongwa HP, Carries S, Gqaleni N (2010) Lactic acid fermentation improves the quality of amahewu, a traditional South African maize-based porridge. Food Chem 122(3):656–661

Chen LS, Ma Y, Maubois JL, He SH, Chen LJ, Li HM (2010) Screening for the potential probiotic yeast strains from raw milk to assimilate cholesterol. Dairy Sci Technol 90(5):537–548

Chen M, Sun Q, Giovannucci E, Mozaffarian D, Manson JE, Willett WC, Hu FB (2014) Dairy consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. BMC Med 12(1):215

Chen Y, Aorigele C, Wang C, Simujide H, Yang S (2015) Screening and extracting mycocin secreted by yeast isolated from koumiss and their antibacterial effect. J Food Nutr Res 3(1):52–56

Cheng H (2010) Volatile flavor compounds in yogurt: a review. Crit Rev Food Sci Nutr 50(10):938–950

Chilton SN, Burton JP, Reid G (2015) Inclusion of fermented foods in food guides around the world. Forum Nutr 7(1):390–404

Choi IK, Jung SH, Kim BJ, Park SY, Kim J, Han HU (2003) Novel *Leuconostoc citreum* starter culture system for the fermentation of kimchi, a fermented cabbage product. Antonie Van Leeuwenhoek 84(4):247–253

Cogan TM, Beresford TP, Steele J, Broadbent J, Shah NP, Ustunol Z (2007) Invited review: advances in starter cultures and cultured foods. J Dairy Sci 90(9):4005–4021

Coppola S, Mauriello G, Aponte M, Moschetti G, Villani F (2000) Microbial succession during ripening of Naples-type salami, a southern Italian fermented sausage. Meat Sci 56(4):321–329

Corbo MR, Lanciotti R, Albenzio M, Sinigaglia M (2001) Occurrence and characterization of yeasts isolated from milks and dairy products of Apulia region. Int J Food Microbiol 69(1):147–152

Corona-González RI, Ramos-Ibarra JR, Gutiérrez-González P, Pelayo-Ortiz C, Guatemala-Morales GM, Arriola-Guevara E (2013) The use of response surface methodology to evaluate the fermentation conditions in the production of tepache. Revista Mexicana de Ingeniería Química 12(1):19–28

Corsetti A, Perpetuini G, Schirone M, Tofalo R, Suzzi G (2012) Application of starter cultures to table olive fermentation: an overview on the experimental studies. Front Microbiol 3:1–6

Coton E, Coton M, Levert D, Casaregola S, Sohier D (2006) Yeast ecology in French cider and black olive natural fermentations. Int J Food Microbiol 108(1):130–135

Coulin P, Farah Z, Assanvo J, Spillmann H, Puhan Z (2006) Characterisation of the microflora of attiéké, a fermented cassava product, during traditional small scale preparation. Int J Food Microbiol 106(2):131–136

Crafack M, Mikkelsen MB, Saerens S et al (2013) Influencing cocoa flavour using *Pichia kluyveri* and *Kluyveromyces marxianus* in a defined mixed starter culture for cocoa fermentation. Int J Food Microbiol 167:103–116

Dalié DKD, Deschamps AM, Richard-Forget F (2010) Lactic acid bacteria–potential for control of mould growth and mycotoxins: a review. Food Control 21(4):370–380

Daniel HM, Vrancken G, Takrama JF, Camu N, De Vos P, De Vuyst L (2009) Yeast diversity of Ghanaian cocoa bean heap fermentations. FEMS Yeast Res 9:774–783

Daniel HM, Moons MC, Huret S, Vrancken G, De Vuyst L (2011) *Wickerhamomyces anomalus* in the sourdough microbial ecosystem. Antonie Van Leeuwenhoek 99(1):63–73

De Castro A, Montaño A, Casado FJ, Sánchez AH, Rejano L (2002) Utilization of *Enterococcus casseliflavus* and *Lactobacillus pentosus* as starter cultures for Spanish-style green olive fermentation. Food Microbiol 19(6):637–644

De Vuyst L (2000) Technology aspects related to the application of functional starter cultures. Food Technol Biotechnol 38(2):105–112

De Wit M, Osthoff G, Viljoen BC, Hugo A (2005) A comparative study of lipolysis and proteolysis in cheddar cheese and yeast-inoculated cheddar cheeses during ripening. Enzym Microb Technol 37(6):606–616

Drider D, Bekal S, Prevost H (2004) Genetic organization and expression of citrate permease in lactic acid bacteria. Genet Mol Res 3:273–281

Ebner S, Smug LN, Kneifel W, Salminen SJ, Sanders ME (2014) Probiotics in dietary guidelines and clinical recommendations outside the European Union. World J Gastroenterol: WJG 20(43):16095

Elmacı SB, Tokatlı M, Dursun D, Özçelik F, Şanlıbaba P (2015) Phenotypic and genotypic identification of lactic acid bacteria isolated from traditional pickles of the Çubuk region in Turkey. Folia Microbiol 60(3):241–251

Erten H, Ağirman B, Gündüz CPB, Çarşanba E, Sert S, Bircan S, Tangüler H (2014) Importance of yeasts and lactic acid bacteria in food processing. In: Food processing: strategies for quality assessment. Springer, New York, pp 351–378

Fadda ME, Viale S, Deplano M, Pisano MB, Cosentino S (2010) Characterization of yeast population and molecular fingerprinting of *Candida zeylanoides* isolated from goat's milk collected in Sardinia. Int J Food Microbiol 136(3):376–380

Faria-Oliveira F, Diniz RH, Godoy-Santos F, Piló FB, Mezadri H, Castro IM, Brandão RL (2015) The role of yeast and lactic acid bacteria in the production of fermented beverages in South America. In: Food production and industry. InTech

Feijoo-Siota L, Blasco L, Luis Rodriguez-Rama J, Barros-Velázquez J, de Miguel T, Sánchez-Pérez A, Villa G, T. (2014) Recent patents on microbial proteases for the dairy industry. Recent Adv DNA Gene Seq (Formerly Recent Patents DNA Gene Seq) 8(1):44–55

Ferreira AD, Viljoen BC (2003) Yeasts as adjunct starters in matured cheddar cheese. Int J Food Microbiol 86(1):131–140

Fleet GH (2003) Yeast interactions and wine flavour. Int J Food Microbiol 86(1):11–22

Foerst P, Santivarangkna C (2014) In: Holzapfel W (ed) Advances in starter culture technology: focus on drying processes. Advances in fermented foods and beverages: improving quality, technologies and health benefits. Woodhead Publishing, Cambridge, UK, pp 249–270

Fontán MCG, Martínez S, Franco I, Carballo J (2006) Microbiological and chemical changes during the manufacture of kefir made from cows' milk, using a commercial starter culture. Int Dairy J 16(7):762–767

Galle S, Schwab C, Arendt E, Gänzle M (2010) Exopolysaccharide-forming *Weissella* strains as starter cultures for sorghum and wheat sourdoughs. J Agric Food Chem 58(9):5834–5841

Garofalo C, Osimani A, Milanović V, Aquilanti L, De Filippis F, Stellato G, Clementi F (2015) Bacteria and yeast microbiota in milk kefir grains from different Italian regions. Food Microbiol 49:123–133

Geis A (2003) Perspectives of genetic engineering of bacteria used in food fermentations. In: Heller KJ (ed) Genetically engineered food: methods and detection. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp 100–118

González-Quijano GK, Dorantes-Alvarez L, Hernández-Sánchez H, Jaramillo-Flores ME, de Jesús Perea-Flores M, Vera-Ponce de León A, Hernández-Rodríguez C (2014) Halotolerance and survival kinetics of lactic acid bacteria isolated from jalapeño pepper (Capsicum annuum L.) fermentation. J Food Sci 79(8):M1545–M1553

Greppi A, Krych Ł, Costantini A, Rantsiou K, Hounhouigan DJ, Arneborg N et al (2015) Phytase-producing capacity of yeasts isolated from traditional African fermented food products and PHYPk gene expression of *Pichia kudriavzevii* strains. Int J Food Microbiol 205:81–89

Gupta C, Prakash D, Gupta S (2015) A biotechnological approach to microbial based perfumes and flavours. J Microbiol Exp 2(1):00034

Hammes WP, Brandt MJ, Francis KL, Rosenheim J, Seitter MF, Vogelmann SA (2005) Microbial ecology of cereal fermentations. Trends Food Sci Technol 16(1):4–11

Hansen EB (2002) Commercial bacterial starter cultures for fermented foods of the future. Int J Food Microbiol 78(1):119–131

Hayakawa K, Kimura M, Kasaha K, Matsumoto K, Sansawa H, Yamori Y (2004) Effect of a γ-aminobutyric acid-enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar–Kyoto rats. Br J Nutr 92(3):411–417

Hjortmo SB, Hellström AM, Andlid TA (2008) Production of folates by yeasts in Tanzanian fermented togwa. FEMS Yeast Res 8(5):781–787

Ho VTT, Zhao J, Fleet GH (2014) Yeasts are essential for cocoa bean fermentation. Int J Food Microbiol 174:72–87

Høier E, Janzen T, Henriksen CM, Rattray F, Brockmann E, Johansen E (1999) The production, application and action of lactic cheese starter cultures. In: Law BA (ed) Technology of cheese making. Sheffild Academic Press, Sheffild, pp 99–131

Holzapfel WH (2002) Appropriate starter culture technologies for small-scale fermentation in developing countries. Int J Food Microbiol 75(3):197–212

Hou J, Hannon JA, McSweeney PL, Beresford TP, Guinee TP (2017) Effect of galactose metabolising and non-metabolising strains of *Streptococcus thermophilus* as a starter culture adjunct on the properties of cheddar cheese made with low or high pH at whey drainage. Int Dairy J 65:44–55

Hugenholtz J, Kleerebezem M, Starrenburg M, Delcour J, de Vos W, Hols P (2000) *Lactococcus lactis* as a cell factory for high-level diacetyl production. Appl Environ Microbiol 66(9):4112–4114

Hurtado A, Reguant C, Esteve-Zarzoso B, Bordons A, Rozès N (2008) Microbial population dynamics during the processing of Arbequina table olives. Food Res Int 41(7):738–744

Hutkins RW (ed) (2006) Introduction in microbiology and technology of fermented foods, Blackwell Publishing, Ames, Iowa, USA

Jayani RS, Saxena S, Gupta R (2005) Microbial pectinolytic enzymes: a review. J Food Biochem 40:2931–2944

Jayaram VB, Cuyvers S, Lagrain B, Verstrepen KJ, Delcour JA, Courtin CM (2013) Mapping of *Saccharomyces cerevisiae* metabolites in fermenting wheat straight-dough reveals succinic acid as pH-determining factor. Food Chem 136(2):301–308

Jayaram VB, Cuyvers S, Verstrepen KJ, Delcour JA, Courtin CM (2014) Succinic acid in levels produced by yeast (*Saccharomyces cerevisiae*) during fermentation strongly impacts wheat bread dough properties. Food Chem 151:421–428

Kapsenberg ML (2003) Dendritic-cell control of pathogen-driven T-cell polarization. Nat Rev Immunol 3(12):984–993

Kariluoto S, Vahteristo L, Salovaara H, Katina K, Liukkonen KH, Piironen V (2004) Effect of baking method and fermentation on folate content of rye and wheat breads. Cereal Chem 81(1):134–139

Kariluoto S, Aittamaa M, Korhola M, Salovaara H, Vahteristo L, Piironen V (2006) Effects of yeasts and bacteria on the levels of folates in rye sourdoughs. Int J Food Microbiol 106(2):137–143

Katina K, Poutanen K (2013) Nutritional aspects of cereal fermentation with lactic acid bacteria and yeast. In: Handbook on sourdough biotechnology. Springer, USA, pp 229–244

Katina K, Laitila A, Juvonen R, Liukkonen KH, Kariluoto S, Piironen V, Poutanen K (2007) Bran fermentation as a means to enhance technological properties and bioactivity of rye. Food Microbiol 24(2):175–186

Kim JY, Lee MY, Ji GE, Lee YS, Hwang KT (2009) Production of γ-aminobutyric acid in black raspberry juice during fermentation by *Lactobacillus brevis* GABA100. Int J Food Microbiol 130(1):12–16

Kim B, Hong VM, Yang J, Hyun H, Im JJ, Hwang J, Kim JE (2016) A review of fermented foods with beneficial effects on brain and cognitive function. Prev Nutr Food Sci 21(4):297

Kimaryo VM, Massawe GA, Olasupo NA, Holzapfel WH (2000) The use of a starter culture in the fermentation of cassava for the production of "kivunde", a traditional Tanzanian food product. Int J Food Microbiol 56(2):179–190

Kingcha Y, Tosukhowong A, Zendo T, Roytrakul S, Luxananil P, Chareonpornsook K, Visessanguan W (2012) Anti-listeria activity of *Pediococcus pentosaceus* BCC 3772 and application as starter culture for Nham, a traditional fermented pork sausage. Food Control 25(1):190–196

Kleerebezem M, Kuipers OP, Smid EJ (2017) Lactic acid bacteria—a continuing journey in science and application. FEMS Microbiol Rev 41(Supp_1):S1–S2

Kopermsub P, Yunchalard S (2010) Identification of lactic acid bacteria associated with the production of plaa-som, a traditional fermented fish product of Thailand. Int J Food Microbiol 138(3):200–204

Korakli M, Gänzle MG, Vogel RF (2002) Metabolism by bifidobacteria and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by *Lactobacillus sanfranciscensis*. J Appl Microbiol 92(5):958–965

Kumar P, Chatli MK, Verma AK, Mehta N, Malav OP, Kumar D, Sharma N (2017) Quality, functionality, and shelf life of fermented meat and meat products: a review. Crit Rev Food Sci Nutr 57(13):2844–2856

Kumura H, Tanoue Y, Tsukahara M, Tanaka T, Shimazaki K (2004) Screening of dairy yeast strains for probiotic applications. J Dairy Sci 87(12):4050–4056

Lacerda CHF, Hayashi C, Soares CM, Boscolo WR, Kavata LCB (2005) Replacement of corn Zea mays L. by cassava *Manihot esculenta* crants meal in grass-carp *Ctenopharyngodon idella* fingerlings diets. Acta Sci Anim Sci 27(2):241–245

Ladero V, del Rio B, Linares DM, Fernandez M, Mayo B, Martin MC, Alvarez MA (2014) Genome sequence analysis of the biogenic amine-producing strain *Lactococcus lactis* subsp. *cremoris* CECT 8666 (formerly GE2-14). Genome Announc 2(5):e01088–e01014

Lamontanara A, Orrù L, Cattivelli L, Russo P, Spano G, Capozzi V (2014) Genome sequence of *Oenococcus oeni* OM27, the first fully assembled genome of a strain isolated from an Italian wine. Genome Announc 2(4):e00658–e00614

Landete JM (2017) A review of food-grade vectors in lactic acid bacteria: from the laboratory to their application. Crit Rev Biotechnol 37(3):296–308

LeBlanc JG, Milani C, de Giori GS, Sesma F, Van Sinderen D, Ventura M (2013) Bacteria as vitamin suppliers to their host: a gut microbiota perspective. Curr Opin Biotechnol 24(2):160–168

Lee JS, Heo GY, Lee JW, Oh YJ, Park JA, Park YH, Ahn JS (2005) Analysis of kimchi microflora using denaturing gradient gel electrophoresis. Int J Food Microbiol 102(2):143–150

Lefeber T, Papalexandratou Z, Gobert W, Camu N, De Vuyst L (2012) On-farm implementation of a starter culture for improved cocoa bean fermentation and its influence on the flavour of chocolates produced thereof. Food Microbiol 30:379–392

Leite AMO, Mayo B, Rachid CTCC, Peixoto RS, Silva JT, Paschoalin VMF, Delgado S (2012) Assessment of the microbial diversity of Brazilian kefir grains by PCR-DGGE and pyrosequencing analysis. Food Microbiol 31(2):215–221

Leroy F, De Vuyst L (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol 15(2):67–78

Leroy F, Verluyten J, De Vuyst L (2006) Functional meat starter cultures for improved sausage fermentation. Int J Food Microbiol 106(3):270–285

Limsowtin GKY, Powell IB, Parente E (1996) Types of starters. In: Cogan TM, Accolas JE (eds) Dairy starter cultures. VCH, New York, pp 101–129

London LEE, Chaurin V, Auty MAE, Fenelon MA, Fitzgerald GF, Ross RP, Stanton C (2015) Use of *Lactobacillus mucosae* DPC 6426, an exopolysaccharide-producing strain, positively influences the techno-functional properties of yoghurt. Int Dairy J 40:33–38

Lortal S, Chapot-Chartier MP (2005) Role, mechanisms and control of lactic acid bacteria lysis in cheese. Int Dairy J 15(6):857–871

Magalhães KT, Pereira GDM, Dias DR, Schwan RF (2010) Microbial communities and chemical changes during fermentation of sugary Brazilian kefir. World J Microbiol Biotechnol 26(7):1241–1250

Malisorn C, Suntornsuk W (2008) Optimization of β-carotene production by *Rhodotorula glutinis* DM28 in fermented radish brine. Bioresour Technol 99(7):2281–2287

Marco ML, Heeney D, Binda S, Cifelli CJ, Cotter PD, Foligne B, Smid EJ (2017) Health benefits of fermented foods: microbiota and beyond. Curr Opin Biotechnol 44:94–102

Martín B, Jofré A, Garriga M, Pla M, Aymerich T (2006) Rapid quantitative detection of *Lactobacillus sakei* in meat and fermented sausages by real-time PCR. Appl Environ Microbiol 72(9):6040–6048

Masoud W, Jespersen L (2006) Pectin degrading enzymes in yeasts involved in fermentation of *Coffea arabica* in East Africa. Int J Food Microbiol 110:291–296

Matthews A, Grimaldi A, Walker M, Bartowsky E, Grbin P, Jiranek V (2004) Lactic acid bacteria as a potential source of enzymes for use in vinification. Appl Environ Microbiol 70(10):5715–5731

Maturano YP, Nally MC, Toro ME, De Figueroa LIC, Combina M, Vazquez F (2012) Monitoring of killer yeast populations in mixed cultures: influence of incubation temperature of microvinifications samples. World J Microbiol Biotechnol 28(11):3135–3142

Mauriello G, Casaburi A, Blaiotta G, Villani F (2004) Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy. Meat Sci 67(1):149–158

McGovern PE, Zhang J, Tang J, Zhang Z, Hall GR, Moreau RA, Cheng G (2004) Fermented beverages of pre-and proto-historic China. Proc Natl Acad Sci U S A 101(51):17593–17598

McSweeney PL, Sousa MJ (2000) Biochemical pathways for the production of flavour compounds in cheeses during ripening: a review. Lait 80(3):293–324

Michaylova M, Minkova S, Kimura K, Sasaki T, Isawa K (2007) Isolation and characterization of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* from plants in Bulgaria. FEMS Microbiol Lett 269(1):160–169

Mohammadi R, Sohrabvandi S, Mohammad Mortazavian A (2012) The starter culture characteristics of probiotic microorganisms in fermented milks. Eng Life Sci 12:399–409

Mokoena MP (2017) Lactic acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini-review. Molecules 22(8):1255

Montaño A, Sánchez AH, Casado FJ, de Castro A, Rejano L (2003) Chemical profile of industrially fermented green olives of different varieties. Food Chem 82:297–302

Montel MC, Buchin S, Mallet A, Delbes-Paus C, Vuitton DA, Desmasures N, Berthier F (2014) Traditional cheeses: rich and diverse microbiota with associated benefits. Int J Food Microbiol 177:136–154

Moslehi-Jenabian S, Lindegaard L, Jespersen L (2010) Beneficial effects of probiotic and food borne yeasts on human health. Forum Nutr 2(4):449–473

Mukdsi MCA, Haro C, González SN, Medina RB (2013) Functional goat milk cheese with feruloyl esterase activity. J Funct Foods 5(2):801–809

Newberry MP, Phan-Thien N, Larroque OR, Tanner RI, Larsen NG (2002) Dynamic and elongation rheology of yeasted bread doughs. Cereal Chem 79(6):874

Nguyen TTT, Loiseau G, Icard-Vernière C, Rochette I, Trèche S, Guyot JP (2007) Effect of fermentation by amylolytic lactic acid bacteria, in process combinations, on characteristics of rice/soybean slurries: a new method for preparing high energy density complementary foods for young children. Food Chem 100(2):623–631

Nielsen DS, Teniola OD, Ban-Koffi L, Owusu M, Andersson TS, Holzapfel WH (2007) The microbiology of Ghanaian cocoa fermentations analysed using culture-dependent and culture-independent methods. Int J Food Microbiol 114:168–186

Nout MR (2003) 17 Traditional fermented products from Africa, Latin America and Asia. In: Boekhout T, Robert V (eds) Yeasts in Food-Beneficial and Detrimental Aspects. Behr's-Verlag GmbH & Co. KG, Hamburg, Germany pp 451–473

Ogunremi OR, Sanni AI, Agrawal R (2015) Probiotic potentials of yeasts isolated from some cereal-based Nigerian traditional fermented food products. J Appl Microbiol 119(3): 797–808

Oguntoyinbo FA, Tourlomousis P, Gasson MJ, Narbad A (2011) Analysis of bacterial communities of traditional fermented West African cereal foods using culture independent methods. Int J Food Microbiol 145(1):205–210

Ong L, Henriksson A, Shah NP (2006) Development of probiotic cheddar cheese containing *Lactobacillus acidophilus, Lb. casei, Lb. paracasei* and *Bifidobacterium* spp. and the influence of these bacteria on proteolytic patterns and production of organic acid. Int Dairy J 16(5):446–456

Padilla B, Manzanares P, Belloch C (2014) Yeast species and genetic heterogeneity within *Debaryomyces hansenii* along the ripening process of traditional ewes' and goats' cheeses. Food Microbiol 38:160–166

Páez-Lerma JB, Arias-García A, Rutiaga-Quiñones OM, Barrio E, Soto-Cruz NO (2013) Yeasts isolated from the alcoholic fermentation of *Agave duranguensis* during mezcal production. Food Biotechnol 27(4):342–356

Papalexandratou Z, De Vuyst L (2011) Assessment of the yeast species composition of cocoa bean fermentations in different cocoa-producing regions using denaturing gradient gel electrophoresis. FEMS Yeast Res 11(7):564–574

Paramithiotis S, Chouliaras Y, Tsakalidou E, Kalantzopoulos G (2005) Application of selected starter cultures for the production of wheat sourdough bread using a traditional three-stage procedure. Process Biochem 40(8):2813–2819

Parente E, Cogan TM (2004) Starter cultures: general aspects. In: Fox PF, McSweeney PLH, Cogan TM, Guinee TP (eds) Cheese: chemistry, physics and microbiology, 3rd edn. Elsevier, London, pp 123–148

Park KB, Oh SH (2006) Isolation and characterization of *Lactobacillus buchneri* strains with high γ-aminobutyric acid producing capacity from naturally aged cheese. Food Sci Biotechnol 15:86–90

Patel A, Shah N, Prajapati JB (2013) Biosynthesis of vitamins and enzymes in fermented foods by lactic acid bacteria and related genera-A promising approach. Croat J Food Sci Technol 5(2):85–91

Patrignani F, Lucci L, Vallicelli M, Guerzoni ME, Gardini F, Lanciotti R (2007) Role of surface-inoculated *Debaryomyces hansenii* and *Yarrowia lipolytica* strains in dried fermented sausage manufacture. Part 1: evaluation of their effects on microbial evolution, lipolytic and proteolytic patterns. Meat Sci 75:676–686

Petrova PM, Petrov KK (2011) Antimicrobial activity of starch degrading *Lactobacillus* strains isolated from boza. Biotechnol Biotechnol Equip 25:114–116

Petrova P, Petrov K, Stoyancheva G (2013) Starch-modifying enzymes of lactic acid bacteria–structures, properties, and applications. Starch-Stärke 65(1–2):34–47

Pistarino E, Aliakbarian B, Casazza AA, Paini M, Cosulich ME, Perego P (2013) Combined effect of starter culture and temperature on phenolic compounds during fermentation of Taggiasca black olives. Food Chem 138:2043–2049

Poutanen K, Flander L, Katina K (2009) Sourdough and cereal fermentation in a nutritional perspective. Food Microbiol 26(7):693–699

Powell IB, Broome MC, Limsowtin GKY (2011) Cheese: starter cultures: specific properties. In: Fuquay JW, Fox PF, McSweeney PLH (eds) Encyclopedia of dairy sciences. Elsevier Academic, Amsterdam, pp 559–566

Psani M, Kotzekidou P (2006) Technological characteristics of yeast strains and their potential as starter adjuncts in Greek-style black olive fermentation. World J Microbiol Biotechnol 22(12):1329–1336

Purriños L, Carballo J, Lorenzo JM (2013) The influence of *Debaryomyces hansenii*, *Candida deformans* and *Candida zeylanoides* on the aroma formation of dry-cured 'lacón'. Meat Sci 93:344–350

Rad AH, Khosroushahi AY, Khalili M, Jafarzadeh S (2016) Folate bio-fortification of yoghurt and fermented milk: a review. Dairy Sci Technol 96(4):427–441

Rai AK, Jeyaram K (2015) Health benefits of functional proteins in fermented foods. In: Tamang JP (ed) Health benefits of fermented foods and beverages. CRC Press, London, New York, pp 455–474

Ray RC, Sivakumar PS (2009) Traditional and novel fermented foods and beverages from tropical root and tuber crops. Int J Food Sci Technol 44(6):1073–1087

Ray M, Ghosh K, Singh S, Mondal KC (2016) Folk to functional: an explorative overview of rice-based fermented foods and beverages in India. J Ethn Foods 3(1):5–18

Reddy G, Altaf MD, Naveena BJ, Venkateshwar M, Kumar EV (2008) Amylolytic bacterial lactic acid fermentation—a review. Biotechnol Adv 26(1):22–34

Rhee SJ, Lee JE, Lee CH (2011) Importance of lactic acid bacteria in Asian fermented foods. Microb Cell Factories 10(1):S5

Romano P, Capece A, Jespersen L (2006) Taxonomic and ecological diversity of foods and beverage yeasts. In: Querol A, Fleet GH (eds) Yeasts in food and beverages. Springer, Berlin, pp 13–54

Ruiz-Rodríguez L, Bleckwedel J, Eugenia Ortiz M, Pescuma M, Mozzi F (2017) Lactic Acid Bacteria. In: Wittmann C, Liao JC (eds) Industrial Biotechnology: Microorganisms. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp 395–451

Rul F, Zagorec M, Champomier-Vergès MC (2013) Lactic acid bacteria in fermented foods. In: Proteomics in foods. Springer, USA, pp 261–283

Saarela M, Mogensen G, Fondén R, Mättö J, Mattila-Sandholm T (2000) Probiotic bacteria: safety, functional and technological properties. J Biotechnol 84(3):197–215

Saithong P, Panthavee W, Boonyaratanakornkit M, Sikkhamondhol C (2010) Use of a starter culture of lactic acid bacteria in plaa-som, a Thai fermented fish. J Biosci Bioeng 110(5):553–557
Sanchez AH, Rejano L, Montano A, de Castro A (2001) Utilization at high pH of starter cultures of lactobacilli for Spanish-style green olive fermentation. Int J Food Microbiol 67(1–2):115–122
Sánchez-Molinero F, Arnau J (2008) Effect of the inoculation of a starter culture and vacuum packaging during the resting stage on sensory traits of dry-cured ham. Meat Sci 80:1074–1080
Santivarangkna C, Kulozik U, Foerst P (2007) Alternative drying processes for the industrial preservation of lactic acid starter cultures. Biotechnol Prog 23(2):302–315
Saubade F, Hemery YM, Guyot JP, Humblot C (2017) Lactic acid fermentation as a tool for increasing the folate content of foods. Crit Rev Food Sci Nutr 57:3894–3910
Sauer U (2001) Evolutionary engineering of industrially important microbial phenotypes. Adv Biochem Eng Biotechnol 73:129–170
Selhub EM, Logan AC, Bested AC (2014) Fermented foods, microbiota, and mental health: ancient practice meets nutritional psychiatry. J Physiol Anthropol 33(1):2
Seok JH, Park KB, Kim YH, Bae MO, Lee MK, Oh SH (2008) Production and characterization of kimchi with enhanced levels of γ-aminobutyric acid. Food Sci Biotechnol 17(5):940–946
Sicard D, Legras JL (2011) Bread, beer and wine: yeast domestication in the *Saccharomyces* sensu stricto complex. C R Biol 334(3):229–236
Silva CF, Vilela DM, de Souza Cordeiro C, Duarte WF, Dias DR, Schwan RF (2013) Evaluation of a potential starter culture for enhance quality of coffee fermentation. World J Microbiol Biotechnol 29:235–247
Singracha P, Niamsiri N, Visessanguan W, Lertsiri S, Assavanig A (2017) Application of lactic acid bacteria and yeasts as starter cultures for reduced-salt soy sauce (moromi) fermentation. LWT Food Sci Technol 78:181–188
Smid EJ, Kleerebezem M (2014) Production of aroma compounds in lactic fermentations. Annu Rev Food Sci Technol 5:313–326
Smit G, Smit BA, Engels WJ (2005) Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. FEMS Microbiol Rev 29(3):591–610
Sodini I, Lucas A, Oliveira MN, Remeuf F, Corrieu G (2002) Effect of milk base and starter culture on acidification, texture, and probiotic cell counts in fermented milk processing. J Dairy Sci 85(10):2479–2488
Speranza B, Bevilacqua A, Corbo MR, Sinigaglia M (eds) (2016) Starter cultures in food production. Wiley, Hoboken
Steinkraus KH (2002) Fermentations in world food processing. Compr Rev Food Sci Food Saf 1(1):23–32
Takeshima K, Yamatsu A, Yamashita Y, Watabe K, Horie N, Masuda K, Kim M (2014) Subchronic toxicity evaluation of γ-aminobutyric acid (GABA) in rats. Food Chem Toxicol 68:128–134
Tamang JP, Fleet GH (2009) Yeasts diversity in fermented foods and beverages. In: Yeast biotechnology: diversity and applications. Springer, Netherlands, pp 169–198
Tamang JP, Kailasapathy K (eds) (2010) Fermented foods and beverages of the world. CRC Press, New York
Tamang JP, Tamang B, Schillinger U, Franz CM, Gores M, Holzapfel WH (2005) Identification of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. Int J Food Microbiol 105(3):347–356
Tamang JP, Watanabe K, Holzapfel WH (2016) Diversity of microorganisms in global fermented foods and beverages. Front Microbiol 7:377
Tamime AY, Thomas L (eds) (2017) Probiotic dairy products. Wiley, Hoboken
Tapsell LC (2015) Fermented dairy food and CVD risk. Br J Nutr 113(S2):S131–S135
Taskila S (2016) Industrial production of starter cultures. In: Speranza B, Bevilacqua A, Corbo MR, Sinigaglia M (eds.) Starter Cultures in Food Production. John Wiley & Sons., Chichester, United States, pp 79–100
Teniola OD, Odunfa SA (2001) The effects of processing methods on the levels of lysine, methionine and the general acceptability of ogi processed using starter cultures. Int J Food Microbiol 63(1–2):1–9

Tornadijo ME, Fresno JM, Sarmiento RM, Carballo J (1998) Study of the yeasts during the ripening process of Armada cheeses from raw goat's milk. Lait 78(6):647–659
Treven P, Trmčić A, Matijašić BB, Rogelj I (2014) Improved draft genome sequence of probiotic strain *Lactobacillus gasseri* K7. Genome Announc 2(4):e00725–e00714
Tsai JS, Lin YS, Pan BS, Chen TJ (2006) Antihypertensive peptides and γ-aminobutyric acid from prozyme 6 facilitated lactic acid bacteria fermentation of soymilk. Process Biochem 41(6):1282–1288
Udomsil N, Rodtong S, Choi YJ, Hua Y, Yongsawatdigul J (2011) Use of *Tetragenococcus halophilus* as a starter culture for flavor improvement in fish sauce fermentation. J Agric Food Chem 59(15):8401–8408
Valyasevi R, Rolle RS (2002) An overview of small-scale food fermentation technologies in developing countries with special reference to Thailand: scope for their improvement. Int J Food Microbiol 75(3):231–239
Viljoen BC (2001) The interaction between yeasts and bacteria in dairy environments. Int J Food Microbiol 69(1):37–44
Viljoen B (2006) Yeast ecological interactions. Yeast' yeast, yeast' bacteria, yeast' fungi interactions and yeasts as biocontrol agents. In: Yeasts in food and beverages. Springer, Berlin, pp 83–110
Vrancken G, De Vuyst L, Van der Meulen R, Huys G, Vandamme P, Daniel HM (2010) Yeast species composition differs between artisan bakery and spontaneous laboratory sourdoughs. FEMS Yeast Res 10(4):471–481
Wacher C, Cañas A, Bárzana E, Lappe P, Ulloa M, Owens JD (2000) Microbiology of Indian and Mestizo pozol fermentations. Food Microbiol 17(3):251–256
Wah TT, Walaisri S, Assavanig A, Niamsiri N, Lertsiri S (2013) Co-culturing of *Pichia guilliermondii* enhanced volatile flavor compound formation by *Zygosaccharomyces rouxii* in the model system of Thai soy sauce fermentation. Int J Food Microbiol 160(3):282–289
Welman AD, Maddox IS (2003) Exopolysaccharides from lactic acid bacteria: perspectives and challenges. Trends Biotechnol 21(6):269–274
Wood BJ (2012) Microbiology of fermented foods. Blackie Academic & Professional, London
Xiong T, Li X, Guan Q, Peng F, Xie M (2014) Starter culture fermentation of Chinese sauerkraut: growth, acidification and metabolic analyses. Food Control 41:122–127
Yépez L, Tenea GN (2015) Genetic diversity of lactic acid bacteria strains towards their potential probiotic application. Rom Biotechnol Lett 20(2):10191–10199
Zhang ZY, Liu C, Zhu YZ, Wei YX, Tian F, Zhao GP, Guo XK (2012) Safety assessment of Lactobacillus plantarum JDM1 based on the complete genome. Int J Food Microbiol 153(1):166–170
Zhao L, Li Y, Jiang L, Deng F (2016) Determination of fungal community diversity in fresh and traditional Chinese fermented pepper by pyrosequencing. Microbiol Lett 363(24):fnw273

Chapter 3
Advances in Microbial Fermentation and Fermented Food for Health

Sudhanshu S. Behera, Pankajini Bal, Sushrirekha Das, Smita H. Panda, and Nakulananda Mohanty

Abstract Fermentation has been used long ago for human consumption and food production. Fermentation process is not only beneficial for prolonging the shelf life of foods, beverage, and gruel, but also fermentation can increase the nutritional value in a safe and effective manner. Nowadays, modern fermentation technologies are used for industrial food production; putting these things in mind, it is necessary to identify and optimize naturally occurring microorganisms. It is defined as starter cultures to ensure consistency and commercial viability and also ensure safety, texture, and flavor of the product. The main property is to improve the health and prevent diseases such as colorectal cancer, obesity, and osteoporosis.

Keywords Bacteriocin · Beverage · Disesases · Fermentation · Probiotics

Introduction

Fermented food is universally promoted as healthy and includes a varied group of foods that differ greatly in content of nutrition and energy (Slavin and Lloyd 2012). Popular fermented foods are alcoholic foods/beverages, fermented milk, cheeses, yogurts, sausages, vinegar, pickled vegetables, vegetable protein/amino acid/peptide sauces, leavened and sourdough bread, and paste with meat-like flavors (Liu et al. 2011). In recent years, fermented foods have gained popularity and increased rapidly due to the fact that fermented products fulfil many functional food needs (German et al. 2009). The fermented food is relatively ready to eat, rich in nutrition components, and low in fat contents with a wide diversity of flavor, aroma, and

S. S. Behera
Department of Fisheries and Animal Resource Development, Government of Odisha, Baripada, India

P. Bal · S. Das · S. H. Panda (✉) · N. Mohanty
Department of Zoology, North Orissa University, Baripada, Odisha, India

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_3

texture (Swain et al. 2014). Conventional fermented food shares about one-third of the foods in the human diet. These foods have been subjected to fermentation by probiotic microflora and/or microbial starter, which are either present in or added to the food. The purpose of fermentation is to improve the preservation, taste, texture, or nutritional quality of the food. Fermented food subsidizes the diet of the people in several parts of the world. They provide food security, improve nutrition, destroy unacceptable (toxic) factors in raw products, generate income and occupation, and, moreover, reduce the volume of materials (Praagman et al. 2015).

The demand for fermented food has been boosted since expanded awareness among consumers of the link between health and diet. Health functionality/benefits of fermented food include anti-constipation, anti-obesity, anticancer, colorectal health promotion, cholesterol reduction, anti-oxidative and fibrolytic effect, antiaging properties, and immune and skin health promotion (Slavin and Lloyd 2012). Commodities and foods having safety fermented products are of particular focus as current public dietary recommendations and are directed to improve the overall health of the population (German et al. 2009).

Impact of Diet in Lower-Income Countries

Research has represented that over the past few decades, there is a rapid change in diet. Processed foods, fast foods, and sugar-sweetened beverages have become the mainstream commodities in the name of modernization. For this reason it has become more essential to promote foods which will provide adequate amount of food nutrition along with properties for health promotion and disease prevention, diseases like obesity, diabetes, cardiovascular diseases, cancer, etc. “Westernized” food products are nowadays available in an affordable range throughout the world which has led to a decline in traditional food systems and reducing the practice of fermented food. However, traditional fermented foods due to their integrated role with gut microbiota offer health benefits, with a diet shift from complex carbohydrates to high fat, high proteins, and low fiber. Economic development has led to improved food security and better health; adverse health effects of the nutrition transition includes expanding rate of child obesity.

Microbial Fermentation and Its End Products

The distinct partition of the gastrointestinal tract (GI) is colonized by populations of microorganisms. The predominant species of microbial populations (in the colon) exhibited true symbiosis with the host that is a key to maintain health and well-being of consumers (Roberfroid et al. 2010). A large total of human interference studies has been performed and demonstrated that consumption of certain fermented food/beverages can result in definite changes in the composition of the gut microbiota in line with probiotic concept. However, the probiotic effect is now a well-established scientific fact. The more data are cumulating, it will be acknowledged more in changes in the microbiota's composition, mainly the increase in *bifidobacteria* and/or *lactobacilli*, can be regarded as a marker of intestinal health (Roberfroid et al. 2010). The human colon content metabolically active species of bacteria (colonic microflora) with microbial populations of 10^{11}–10^{12} CFU/g (approx.) (Slavin 2013). The colonic microflora (e.g., lactobacilli and bifidobacteria) having an exclusive potential of saccharolytic metabolism provides considerably to the barrier that prevents pathogenic microflora from assaulting GI-tract (Slavin 2013). *Lactobacillus* has played an important role in the production of fermented products for millennia. They synthesize antimicrobial compounds (bacteriocins) and vitamins and release their contents into fermented products (Panda et al. 2009). It is interesting to note that lactic acid bacteria involved in decreasing serum cholesterol level. Ray and Bhunia (2008) stated that LAB promote to metabolize dietary cholesterol and deconjugate bile salts in the colon and inhibit their reabsorption in the liver and therefore decrease cholesterol levels in the serum.

LAB as a source of probiotics, starter cultures, antimicrobial agents, vitamins, enzymes, and exoplysaccharides (EPS) can satisfy the increasing consumers' demands for natural products and functional foods in relation to human health.

Probiotics

Fermented foods contain viable microorganisms/substances of a suitable concentration and contribute to intestinal microbial balance. The Food and Agriculture Organization (FAO)/World Health Organization (WHO) defined probiotic as "live microbiota, which when administered in adequate quantity confer a health benefit on the host" (Florou-Paneri et al. 2013). The dominated microflora considered as commercial probiotic are mainly of *Lactobacillus* genus with over hundred species recognized, including *Lb. plantarum*, *Lb. acidophilus*, *Lb. rhamnosus*, *Lb. casei*, *Lb. bulgaricus*, *Lb. reuteri*, *Lb. helveticus*, and *Lb. delbrueckii*. However, *Lactobacillus* is generally recognized as safe (GRAS) organisms (Argyri et al. 2013). In the scientific literature, the fermented food contains a population of 10^6–10^7 CFU/g (probiotic counts) and is established as therapeutic quantities (da Cruz et al. 2009). Yogurt has proven to be an excellent vehicle, especially when it contains probiotic microflora. The probiotic microflora is very sensitive to several environmental stresses, including oxygen, temperature, and acidity. In recent years, the derived probiotic therapeutics/drugs are intended to cure, treat, and prevent human diseases (Veena et al. 2010). Moreover, probiotic microflora regulate several functional and physiological activities including stimulation of immune response, production of antimicrobial substances, and counteracting harmful microorganisms. This probiotic microflora suppress food-borne pathogens, such as *Camplobacter* and *Salmonella*, and serve to improve performance and health status of consumers (Sanders 2009).

Starter Culture

The "starter culture" is the microbial preparation introduced to a raw material/substrate to enhance quality of finished fermented product/food (Vijayendra and Halami 2015; Daragh et al. 2017). LAB in the long run has been used as starter cultures, since they can improve nutritional, organoleptic, technological, and shelf-life characteristics in fermented foods and beverages (Li et al. 2006). Panda et al. (2007) prepared lacto-pickle from β-carotene-rich sweet potato by lactic acid fermentation using *Lb. plantarum* as the starter culture. Use of phage-resistant starter cultures also offers a solution for phage contamination in dairy industry (Li et al. 2006). The desired functional starters may be obtained from wild-type organisms or by genetic engineering. This provides better control during fermentation and healthy food for consumers (Li et al. 2006).

Bacteriocins

The bacteriocin is a diverse group of ribosomally synthesized antimicrobial agents originating from mostly bacteria and archaea (Perez et al. 2014). They have bactericidal activity which is used in food preservation and health care (Udhayashree et al. 2012). Commonly bacteriocin is cationic peptides/proteinaceous toxins which display amphiphilic properties and target for their action on bacterial membrane of similar or closely related bacterial strain(s) (Rajaram et al. 2010). Recently, bacteriocins from LAB have created curiosity among researchers as an important and a potential antibiotic for the control of some pathogens and food spoilage organisms (Abdhul et al. 2015). For instance, nisin (bacteriocin) produced by *Lactococcus lactic* subsp. *lactis* from dairy products has been broadly studied and has been approved for use as a food preservative because of its inhibitory effect against food poisoning and non-food-borne isolates (Udompijitkul et al. 2012).

Vitamins

Vitamins are micronutrients that are essential for metabolism of all living organisms (Capozzi et al. 2012). These are essential for normal growth and development of multicellular organisms and mixed up in several necessary functions like cell metabolism and cellular respiration. Humans cannot synthesize most of vitamins, and these must be supplied from other sources/diets. However, some intestinal microflora/bacteria, like LAB, have reported to form vitamins, such as cobalamin (or vitamin B12), menaquinone (vitamin K2), riboflavin, and thiamine, during the production/fermentation of cheeses, yogurt, and other fermented foods (LeBlanc et al. 2013). The vitamins synthesized by LAB vary considerably, being a species-specific or having strain-dependent trait (Turroni et al. 2014). For instance, high levels of B-group vitamins, such as folate and riboflavin, are produced by

LAB-promoted (bifidobacteria) bacterial strain (LeBlanc et al. 2013). Many industrially important LAB such as *Lactobacillus lactis* and *Streptococcus thermophilus* have been reported to synthesize folate (or vitamin B_{11}) (Arena et al. 2014).

Enzymes

The microbial fermentation/microorganism involved in fermented foods produced enzymes to breakdown of complex compounds to simple biomolecules for several biological means. Several enzymes (from *Bacillus* sp.), such as amylase, cellulase, catalase, etc., are responsible for the production of fermented foods. LAB-produced enzymes, in particular amylases, are used in preparation of sourdough bread and to improve its texture and sensory characteristics (Katina et al. 2006). Certain peptides produced by *Lb. lactis* subsp. *cremoris* improve the sensory qualities of cheese. Enzymes also play an important role in wine making. Moreover, the starchy crop, like sweet potato, required saccharification/enzymatic treatment for the conversion of starch into sugar in wine making (Ray et al. 2012; Panda et al. 2015). The alcoholic fermentation of grapes is derived mainly from the activity of the LAB, through the action of their enzymes.

Biologically Active Peptides

The biologically active peptides derived from fermented foods exert physiological effects beyond their nutritional values in humans (Udenigwe and Aluko 2012). Various fermented milk products, such as kefir (Quirós et al. 2005), koumiss (Chen et al. 2010), yogurt (Papadimitriou et al. 2007), fermented camel milk (Moslehishad et al. 2013), and fermented fish products (Harnedy and FitzGerald 2012), form active peptides and have beneficial health effects (Udenigwe and Aluko 2012). The probiotic bacteria, such as *Lactobacillus delbrueckii* and *Streptococcus thermophilus* especially involved in protein producing bioactive peptides and amino acids, during cheese ripening and milk fermentation which have health benefits (Erdmann et al. 2008). For instance, hypertension is a disease increasing in high rate in people living in developing and developed countries. More recently, a great interest has been focused on active peptides that can lower the blood pressure in hypertensive patients (FitzGerald et al. 2004; Udenigwe and Aluko 2012).

Exopolysaccharides *(EPS)*

A number of LAB can produce a variety of long-chain sugar polymers called EPS, which is used as natural thickening agents and gives the product (fermented food) a suitable viscosity (Patel et al. 2014). EPS-producing LAB is especially relevant in yogurt, cheese, sour cream, and other cultured dairy products (Patel et al. 2014). EPS is claimed to have beneficial physiological effects on consumer's health. The presence of EPS promotes to be remained a long time in the gastrointestinal tract for transient colonization of probiotic bacteria (Smith et al. 2017).

Nutritional Composition of Fermented Foods and Their Impact on Human Health

Fermented foods and beverage can enhance the human nutrition by enriching protein, amino acids, essential fatty acids, and vitamin contents. Fermented food products may prevent and treat some diseases, which have safety for human health (Table 3.1). Some fermented food products, which have been patented, are described below.

Milk Product

There are many fermented milk products having undescribed microbiota; however, this microbiota has been characterized using a sequence-based analysis to be useful for human health. Examples of these fermented milk products are *matsoni* (Armenian origin) and *kule naoto* (Kenya origin) (Bokulich et al. 2015). Subsequently, a probiotic fermented camel milk marketed as *shubat* (Kazakh origin) has been currently proved to improve hypoglycemic activity in some type 2 diabetic rats (Manaer et al. 2015). Similarly, another fermented milk beverage (Raabadi) has been investigated

Table 3.1 Different fermented foods and its health benefits

Fermented food/ functional food	Microorganism involved	Health benefit and/or related effect	References
Functional bread	*Lactobacillus lacti* subsp. *cremoris* strain NZ9000	Vitamin B2/riboflavin	Burgess et al. (2004)
Bread and pasta	*L. plantarum*	Vitamin B2/riboflavin	Capozzi et al. (2011)
Sourdough bread	*Weissella cibaria* and *Leuconostoc citreum*	Prevent coelic/celiac disease	Corona et al. (2016)
Dairy and nondairy food products	*L. plantarum* 86, *W. cibaria* 142 and 92 and *P. parvulus* AI1	Antibacterial activity	Patel et al. (2014)
Yoghurt	*Lactobacillus delbrueckii* subsp. *bulgaricus* ϒ10.13 and *Streptococcus thermophilus* ϒ10.7	Reduced the risk of CVD and high BP	Papadimitriou et al. (2007)
Powdered fermented milk	*Lactobacillus helveticus*	Reduces elevated BP	Aihara et al. (2005)

CVD Cardiovascular diseases, *BP* Blood pressure to have hypocholestrol activity, acquiring a good population of probiotic lactobacilli (Yadav et al. 2016). Further, importance is given for safe use of known starter cultures to have useful end products for health benefits. In Kenya, milk is fermented in a goyard to have end products as ethanol and acetyldehyde, which act as etiological factors for esophageal cancer.

Tea

Fermentation of tea is carried out using various probiotic microorganisms to reduce the content of toxic end products such as organic acids and polyphenols. Fermentation with probiotic organisms accumulates vitamins, caffeine, and tannin reduction with increased therapeutic value of bronchodilation (Wu et al. 2010). Fermented tea has proven to have antimicrobial activity against pathogens such as *Salomonella* and *Stayphylococcus*. Kwack et al. (2011) reported a new method for preparing fermented tea with *Bacillus* sp., isolated from Korean fermented foods, which claimed to have improved flavor, safety, and nutritious benefits.

Rice and Soy

Nowadays most vegetarians prefer fermented staple food as an alternative food source from dairy products especially for individuals with lactose intolerance. These needs can be fulfilled from widely grown and staple foods such as soy and rice. Due to the presence of most important photochemical known as isoflavones, these food crops provide mankind a health benefit which may include possible reduction of age-related and hormone-related diseases. When soy is fermented, a chemical conversion of the isoflavone glycoside precursors genistein and daidzein takes place to active isoflavones genistein and daizein, respectively (Borrsen et al. 2012). While doing fermentation of mushrooms grown in fermented soy results in numerous embodiments, those can be used in treatment/relieve a variety of disease-related symptoms. Nair (2011) provided the above overview upon processing of two common fermented products: HAELAN 951® and SoyLac™. These health benefits range from malnutrition and mood-related disorders to metabolic syndrome and chronic diseases. Fermented soy extract is a healthy product which includes a *Lactobacillus* strain and *Saccharomyces* species (Borjab 2006). This fermented soy extracted study gives a warranty on understanding its utility in prevention and/or treatment of inflammatory disease such as cancer, infection, autoimmunity, and asthma. Other plant-based products such as fermented rice and rice bran have been proved to prevent/treat disease. After fermenting rice with *Monascus purpureus* and Red yeast rice is produced and its nutritional profile includes unsaturated fatty acids, sterols, vitamin B complex and monacolins with antioxidant properties.

Even research has shown/proven the treatment of cholesterol, type II diabetes, cardiovascular disease and for cancer prevention also. Zhang et al. (2000) developed methods and compositions of red yeast rice to be used as a dietary supplement which will improve blood lipid dietary panels. Further research is required on red yeast rice bioavailability and clinical outcomes to provide a concrete conclusion with regard to improving human health. In China people used to take red mild rice for many centuries which is another fermented rice product, i.e., *Monascus*. It is used for flavor enhancement and medical treatment. In modern days it is used to prevent major diseases like cardiovascular diseases, cancer, and Alzheimer's disease. Also there is another good news that there is no evident side effects which are major concerns for current medication used to treat age-related diseases.

Biobran is a rice bran functional food product which was developed using water-soluble rice bran a dietary fiber component. This is involved in immune modulation and supposed to enhance health and quality of life. Lastly, metabolite profiles with bioactivity have been found in rice bran fermented with the yeast, *Saccharomyces boulardii*. This new substantiation for the role of rice bran shows photochemical diversity in the presence and absence of fermentation which may have discussions on disease-fighting activities (Ghoneum and Maeda 1996).

Coco Fermentation

Another evidence in analytical food chemistry and molecular biology suggests that the processed cocoa beans and cocoa-based products may contain some substances and chemical compounds of microbial and fungal origin which are highly beneficial to human health (Ivan and Yuriy 2016). A new name, "COCOBIOTA," has been opened into the world of fermentation technology. The "COCOBIOTA" is a definite unit of fungi and bacteria which causes the spontaneous postharvest fermentation of cocoa beans and which have some health effect through various primary and secondary metabolites of fungal-bacterial origin present in cocoa powder and dark chocolate. Adversely, excess cocoa bean fermentation and microbial overgrowth are known to reduce the amounts of antioxidants and flavanols/polyphenols. Further research is required to develop controlled fermentation process of cocoa beans to prevent the loss of essentials (flavanols/polyphenols/antioxidants). Lipopolysaccharides (LPS) from acetic acid bacteria have immune-regulatory activity and affect tumor necrosis factor as well as nitric oxide (NO) production. Similarly, LAB displays a cholesterol lowering and a significant antifungal activity and promotes the formation of short-chain fatty acids.

During cocoa fermentation, LAB produces mannitol, an important prebiotic which modifies the gut microbiota spectrum. Petyaev et al. (2014) suggested that there are different commercial brands of chocolates which contain various bacterial metabolites with well-known biological activities. These biological activities added the full LPS and its fragments, propionic acid and butyrate, and two short-chain fatty acids regulating mitochondrial oxidation. Recent research reveals that *Penicillium citrinum* is a major type of fungi identifiable in fermented cocoa beans, which produces novel thermostable alkaloid and penictrinine-A. It displays significant antitumor and antimetastatic activities. *Aspergillus* family also synthesizes substantial amounts of lovastatin, a powerful inhibitor of cholesterol biosynthesis. These facts suggest that these newly identified fungal metabolites to some extent exert antineoplastic, antiatherogenic, and antibacterial properties of cocoa powder and dark chocolates which have been revealed by many scientists.

Natural Benefits and Curative Properties

Fermented foods protect against development of Western diseases, including diabetes, cardiovascular disease, colon cancer, and obesity (Owsus-Kwarteng et al. 2015). Since that time, research continues on defining fiber and determining the health benefits of fermented food consumption.

Protection Against Gastrointestinal Disorders and Insomnia

Apart from the lactic acid (LA) organisms put in the milk for the purpose of souring, the acid of sour milk and its lactose content have important curative factors (Sawada et al. 1990). Curd is highly beneficial for those patients who are suffering from gastrointestinal disorders, including chronic constipation and diarrhea (Beniwal et al. 2003). Curd also decreases dryness and gas in the stomach by stimulating the secretion of hydrochloric acid (HCl), pepsin, and rennin (Gilliland 1990). Beneficial results have been achieved by the use of buttermilk in case of colitis, gastric ulcer, and dysentery (Danone 2001). Insomnia patients should take plenty of curd and massage it on the head; as a result it will induce sleep (Parvez et al. 2006).

Prevention from Premature Aging, Hepatitis, and Jaundice

Curd has been associated with longevity. Premature old age and decay could be prevented by taking sufficient curd in daily diet (Danone 2001). The excess release of ammonia (NH_3), is one of the major cases of coma in hepatitis which can be avoided by moderate use of curd. LA organisms in the curd work against the formation of NH_3 (Solga 2003).

Protection from Skin Disorder

The use of curd in the form of butter milk is highly beneficial in the treatment of obstinate skin disorders such as psoriasis and eczema (Isolauri et al. 2000). The intense skin irritation generally disappears quickly after the application of buttermilk compresses (Mc Farland 2000; Isolauri 2004). The main benefit is that it is a good source of calcium, vitamins, and minerals which are important for lowering cholesterol and indirectly reduces saturated fat in the diet (Ouwehand et al. 2002). It is also helpful in eradication of vaginal infection, colon cancer, and tumors in animals as studied in rats (Marteau et al. 2001; Sanders and Klaenhammer 2001).

Protection from Celiac Disease

Coeliac disease (CD) or celiac disease (in the USA) is a condition where the person's body reacts to the gliadin fraction of wheat and the prolamins of rye (secalins), barley (hordeins), and oats (avidins) (Murray 1999). CD can only be treated by taking "gluten-free" food or total avoidance of gluten ingestion (Pruska-Kędzior et al. 2008). The "sourdough bread" is a substitute, and it has been proven to improve the texture and palatability of whole grain and fiber-rich products and may stabilize or increase the levels of bioactive compounds considered as "gluten-free" food (Rizzello et al. 2010; Gobbetti et al. 2014; Behera and Ray 2015).

Protection Against Hypertension and Heart Disease

Most of the fermented foods are proved as antihypertensive and are authenticated using animal models and clinical trials (Chen et al. 2009). Milk products having tripeptides (e.g., Ile-Pro-Pro and Val-Pro-Pro) are the best examples of lowering the blood pressure which has been proven in clinical trials (Jauhiainen et al. 2010). Consumption of fermented soybean foods, kefir, calpis, and the Japanese fermented sour milk has shown a hypotensive effect.

Prevention from Cancer

Since the past few decades, cancer has become a serious health issue globally. There are several epidemiological evidences which support the protective role of probiotics against cancer. Fermented milks/dairy products containing probiotic cultures have a protective role against colorectal cancer (CRC) (Saikali et al. 2004). These effects are attributed to the inhibition of mutagenic activity and decrease in several enzymes implicated in the generation of carcinogens, mutagens, or tumor-promoting agents (Kumar et al. 2010). However, evidences as antimutagenic properties of fermented milk/dairy products are not conclusive, and much further research is required to establish this notion.

Protection from Diabetes

Fermented foods contain dietary fiber and fiber intake linked to lower incidence of obesity and diabetes. The complemented diet having the probiotic (i.e., *L. acidophilus* and *L. casei*) low-fat (2–5%) *dahi* extensively delays hyperglycemia, glucose intolerance, hyperinsulinemia, dyslipidemia, and oxidative stress which indicates a lower risk of diabetes and its complications (Yadav et al. 2007).

Relief from Lactose Malabsorption

Fermented foods are processed using probiotic lactic acid bacteria and are substitute for consumers with intolerance or allergy to milk protein (Nychas et al. 2002).

Health Risk of Fermented Food

High levels (>100 mg/kg) of biogenic amines (BAs) (e.g., histamine and tyramine) in the fermented food can have adverse effects to human health (Behera and Ray 2016; Tamang et al. 2016). BAs are low-molecular-weight organic compounds formed by microbial decarboxylation of their precursor amino acids or by transamination of aldehydes and ketones (Tamang et al. 2016), which are present in some fermented foods such as sauerkraut, fish products, cheese, wine, beer, dry sausages, etc. (Zhai et al. 2012).

Safety of Fermented Foods

From safety aspects of fermented foods, researchers claim that fermentation enhances food safety by inhibiting the growth of pathogenic bacteria, toxin degradation, and the improvement of shelf life and digestibility of raw food materials. The preservative nature of LAB species blocks the growth of pathogenic microorganisms by nutrient competition and bacterial inhibition production. Some of the inhibitors include hydrogen peroxide, organic acid, and bacteriocin. Hydrogen peroxide has a strong oxidizing effect on most pathogenic bacteria, whereby lactic and acetic acid are particularly responsible for inhibiting the growth of gram-negative bacteria.

Conservation of foods and beverages ensuing from fermentation has been an effective form of extending the shelf life of foods for millennia. Usually, foods were being preserved through naturally occurring fermentations; however, the recent large-scale production generally now utilizes the use of distinct strain starter systems ensuring consistency and quality in the final product (Fig. 3.1). Ross et al. (2002) studied on the use of LAB species for extensive application and quality improvement

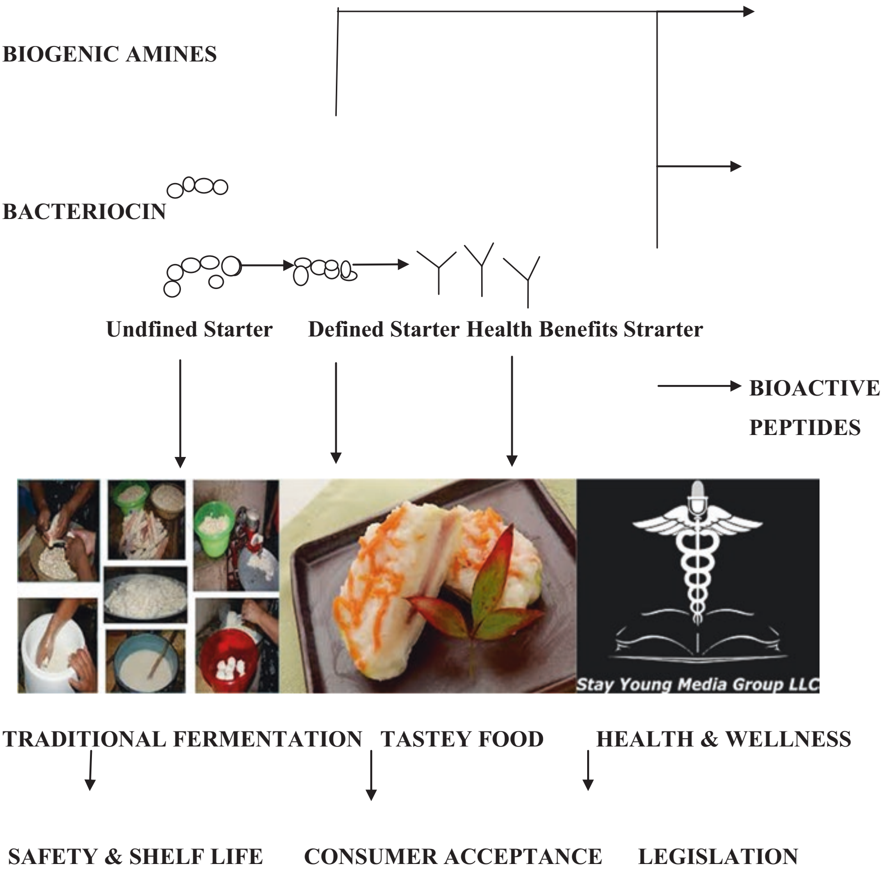


Fig. 3.1 Relationship between fermenting microbes, fermented products, and the consumer (https://www.google.co.in/search?biw=1355&bih=593&tbm=isch&sa=1&q=ethinic+fermented+food+products&oq)

of fermented foods. These microorganisms (LAB) produce a wide diversity range of aggressive primary and secondary metabolites like organic acids, diacetyl, CO_2, and antibiotic such as reuterocyclin produced by *Lb. reuteri*. Moreover, the members of LAB can also produce a wide range of bacteriocin; few of them have action against food-borne pathogens, such as *Listeria monocytogenes* and *Clostridium botulinum*. In fact, the bacteriocin nisin is being used as an useful bio-preservative in some dairy products, wheareas a number of more recently discovered bacteriocin, such as lacticin 3147, revealed an increasing prospective in many food applications. The utilization of such naturally produced rival holds tremendous potential for extension of shelf life and improvement of safety of a variety of fermented foods.

The action of microbes produces a range of metabolites which suppress the growth of undesirable microflora in foodstuffs and hasten the fermentation process. People of both developing and industrialized countries are becoming more aware about the health benefits of such fermented foods, for which the importance is increasing in modern-day life not only for the benefit of preservation and safety but also for their highly appreciated sensory attributes.

Fermentation may be a useful strategy for reducing bacterial contamination of food. This preservation method helps to reduce the dominance of diarrheal diseases. Foods can be produced as well as can be preserved by fermentation technology which is a very economical and low-cost technique. Bomenweg and Wageningen (1994) presented an evaluation report on risk factors correlated with fermented foods, in comparison with fresh or alternatively processed foods. It has been seen that microbial food-borne infections are arising due to consumption of fresh cheese, sausages, fermented fish, and fermented cereals. Microbial food intoxications occurs as mycotoxin contaminates in raw materials, bacterial toxin produces or fungal inoculants produce possible mycotoxin, which is an another risk factor. Additionaly toxic by-products of fermentation may produce ethyl carbamate and biogenic amine-like compounds. From a food processing point of view, food industries face various challenges like the use of contaminated raw materials, lack of pasteurization, and the use of poorly controlled natural fermentations. Safety of fermented foods may reduce in case of suboptimal fermentation starters, insufficient storage, and maturation conditions with consumption without prior cooking. In addition to ensuring adequate processing conditions, the development of non-toxigenic starters with ability to antogonize pathogenic microorganisms and to degrade toxic substances needs continued attention.

Conclusion and Future Prospective

There are few fermented foods and beverages that have health benefits because of the presence of some health-beneficial microorganisms. Innovation process usually involves customer needs and looking at the customer need industries are required to robust implementation of all industrial steps those were involved in the conventional process and adaptation of existing protocol of fermentation technology optimizing the quality and process consistency at all development stages which increases the production scales. The laboratory analysis of testing parameters of fermented food, like organic acid profile, and typical aroma compounds is essential for maintenance of safety, quality, and acceptability to consumers. Additionally, the evaluation of sensory attributes helps in determining some important properties which may control customers. Moreover, validation of health alleged by clinical experiment and animal must be encouraged, so that new fermented food products having well-certified functional microflora may come into sight in the international food market.

References

Abdhul K, Ganesh M, Shanmughapriya S, Vanithamani S, Kanagavel M, Anbarasu K et al (2015) Bacteriocinogenic potential of a probiotic strain Bacillus coagulans [BDU3] from Ngari. Int J Biol Macromol 79:800–806

Aihara K, Kajimoto O, Hirata H, Takahashi R et al (2005) Effect of powdered fermented milk with Lactobacillus helveticus on subjects with high-normal blood pressure or mild hypertension. J American Coll Nutr 24(4):257–265

Arena MP, Russo P, Capozzi V, López P, Fiocco D et al (2014) Probiotic abilities of riboflavin-overproducing Lactobacillus strains: a novel promising application of probiotics. Appl Microbiol Biotechnol 98(17):7569

Argyri AA, Zoumpopoulou G, Karatzas KA, Tsakalidou E et al (2013) Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Food Microbiol 33(2):282–291

Behera SS, Ray RC (2015) Sourdough bread. In: Rosell CM, Bajerska J, El Sheikha AF (eds) Bread fortification for nutrition and health. CRC Press, Boca Raton, pp 53–67

Behera SS, Ray RC (2016) Microbial linamarase in cassava fermentation. In: Ray RC, Rossell CM (eds.) Microbial enzyme technology, ISBN 978-1-4987-4983-1

Beniwal RS, Arena VC, Thomas L, Narla S, Imperiale TF et al (2003) A randomized trial of yoghurt for prevention of antibiotic-associated diarrhoea. Dig Dis Sci 48(10):2077–2082

Bokulich NA, Amiranashvill L, Chitchyan K et al (2015) Microbial biogeography of the transnational fermented milk matsoni. Food Microbiol 50:12–19

Bomenweg HD, Wageningen IN (1994) The Netherlands fermented foods and food safety. Food Res Int 21:291–298

Borjab GS (2006) Lactobacillus delbrueckii sp. Bulgaricus strain and composition. US7901925

Borrsen EC, Henderson AJ, Kumar A et al (2012) Fermented foods: patented approaches and formulations for nutrition supplementation and health promotion. Recent Pat Food Agric 4(2):134–140

Burgess C, O'Connell-Motherway M, Sybesma W et al (2004) Riboflavin production in Lactococcus lactis: potential for in situ production of vitamin-enriched foods. Appl Environ Microbiol 70(10):5769–5777

Capozzi V, Menga V, Digesu AM, De Vita P, van Sinderen D et al (2011) Biotechnological production of vitamin B2-enriched bread and pasta. J Agric Food Chem 59(14):8013–8020

Capozzi V, Russo P, Dueñas MT et al (2012) Lactic acid bacteria producing B-group vitamins: a great potential for functional cereals products. Appl Microbiol Biotechnol 96:1383–1394

Chen ZY, Peng C, Jiao R, Wong YM et al (2009) Anti-hypertensive nutraceuticals and functional foods. J Agric Food Chem 57(11):4485–4499

Chen Y, Wang Z, Chen X, Liu Y, Zhang H, Sun T (2010) Identification of angiotensin I-converting enzyme inhibitory peptides from koumiss, a traditional fermented mare's milk. J Dairy Sci 93(3):884–892

Corona O, Alfonzo A, Ventimiglia G, Nasca A et al (2016) Industrial application of selected lactic acid bacteria isolated from local semolinas for typical sourdough bread production. Food Microbiol 31(59):43–56

da Cruz AG, Buriti FC, de Souza CH et al (2009) Probiotic cheese: hetealth benefits, technological and stability aspects. Trends Food Sci Technol 20(8):344–354

Danone SA (2001) Fermented foods and healthy digestive functions. Danone Publications, John Libbey, Eurotext, France

Daragh H, Ivan S, Elke A et al (2017) Recent advances in fermentation for dairy and health. F1000 Res 6:751

Erdmann K, Cheung BW, Schröder H (2008) The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. J Nutr Biochem 19(10):643–654

FitzGerald RJ, Murray BA, Walsh DJ (2004) Hypotensive peptides from milk proteins. J Nutr 134(4):980–988

Florou-Paneri P, Christaki E, Bonos E (2013) Lactic acid bacteria as source of functional ingredients. In: Lactic acid bacteria-R & D for food, health and livestock purposes. InTech

German JB, Gibson RA, Krauss RM, Nestel P, Lamarche B, Van Staveren WA (2009) A reappraisal of the impact of dairy foods and milk fat on cardiovascular disease risk. Eur J Nutr 48(4):191–203

Ghoneum MH, Maeda H (1996) Immunopotentiator and method of manufacturing the same. 5560914

Gilliland SE (1990) Health and nutritional benefits from lactic acid bacteria. FEMS Microbiol Lett 87(1–2):175–188
Gobbetti M, Rizzello CG, Di Cagno R et al (2014) How the sourdough may affect the functional features of leavened baked goods. Food Microbiol 37:30–40
Harnedy PA, FitzGerald RJ (2012) Bioactive peptides from marine processing waste and shellfish: a review. J Funct Foods 4(1):6–24
Isolauri E (2004) Dietary modification of atopic disease: use of probiotics in the prevention of atopic dermatitis. Curr Allergy Asthma Rep 4:270–275
Isolauri E, Arvola T, Sutas Y et al (2000) Probiotics in the management of atopic eczema. Clin Exp Allergy 30:1604–1610
Ivan MP, Yuriy KB (2016) Cocobiota: implications for human health. J Nutr Metab 2016:7906927
Jauhiainen T, Rönnback M, Vapaatalo H, Wuolle K et al (2010) Long-term intervention with Lactobacillus helveticus fermented milk reduces augmentation index in hypertensive subjects. Eur J Clin Nutr 64(4):424–431
Katina K, Salmenkallio-Marttila M, Partanen R et al (2006) Effects of sourdough and enzymes on staling of high-fibre wheat bread. LWT Food Sci Technol 39(5):479–491
Kumar M, Kumar A, Nagpal R, Mohania D, Behare P et al (2010) Cancer-preventing attributes of probiotics: an update. Int J Food Sci Nutr 61(5):473–496
Kwack I, Lee B, Oh Y et al (2011) Method for preparing fermented tea using Bacillus sp. Strains (as Amended) US201 10250315A
LeBlanc JG, Milani C, de Giori GS, Sesma F et al (2013) Bacteria as vitamin suppliers to their host: a gut microbiota perspective. Curr Opin Biotechnol 24(2):160–168
Li YL, Lu X, Chen XH et al (2006) Lactic acid bacteria as functional starter cultures for the food fermentation industry. Zhonggue Rupin Gongye 34(1):35
Liu SN, Han Y, Zhou ZJ (2011) Lactic acid bacteria in traditional fermented Chinese foods. Food Res Int 44(3):643–651
Manaer T, Yu L, Zhang Y et al (2015) Anti-diabetic effects of shubat in type 2 diabetic rats induced by combination of high-glucose-fat diet and low-dose streptozotocin. J Ethnopharmcol 169:269–274
Marteau P, de Vrese M, Cellier CJ et al (2001) Protection from gastrointestinal diseases with the use of probiotics. Am J Clinic Nutri 73:430–436
Mc Farland LV (2000) Beneficial microbes: health or hazard. Eur J Gastroenterol Hepatol 12:1069–1071
Moslehishad M, Ehsani MR, Salami M, Mirdamadi S et al (2013) The comparative assessment of ACE-inhibitory and antioxidant activities of peptide fractions obtained from fermented camel and bovine milk by Lactobacillus rhamnosus PTCC 1637. Int Dairy J 29(2):82–87
Murray JA (1999) The widening spectrum of celiac disease. American J Clin Nut 69(3):354–353
Nair V (2011) Fermented soy nutritional supplements including mushroom components. US20110206721A1
Nychas GJE, Panagou EZ, Parker ML et al (2002) Microbial colonization of naturally black olives during fermentation and associated biochemical activities in the cover brine. Lett Appl Microbiol 34:173–177
Ouwehand AC, Salminen S, Isolauri E (2002) Probiotics: an overview of beneficial effects Antonie Van Leeuwenhoek. Int J Gen Mole Biol 82:279–289
Owsus-Kwarteng J, Tano-Debrah K, Akabanda F et al (2015) Technological properties and probiotic potential of Lactobacillus fermentum strains isolated from West African fermented millet dough. BMC Microbiol 15:261
Panda SH, Parmanick M, Ray RC (2007) Lactic acid fermentation of sweet potato (Ipomoea batatas L.) into pickles. J Food Process Preserv 31(1):83–101
Panda SH, Naskar SK, Sivakumar PS et al (2009) Lactic acid fermentation of anthocyanin-rich sweet potato (Ipomoea batatas L.) into lacto-juice. Int J Food Sci Technol 44(2):288–296
Panda SK, Panda SH, Swain MR, Ray RC, Kayitesi E (2015) Anthocyanin rich sweet potato (*Ipomoea batatas* L.) beer: technology, biochemical and sensory evaluation. J Food Process Preserv 39(6):3040–3049

Papadimitriou CG, Vafopoulou-Mastrojiannaki A, Silva SV, Gomes AM, Malcata FX, Alichanidis E (2007) Identification of peptides in traditional and probiotic sheep milk yoghurt with angiotensin I-converting enzyme (ACE)-inhibitory activity. Food Chem 105(2):647–656

Parvez S, Malik KA, Ah Kang S et al (2006) Probiotics and their fermented food products are beneficial for health. J Appl Microbiol 100(6):1171–1185

Patel A, Prajapati JB, Holst O et al (2014) Determining probiotic potential of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented food products. Food Biosci 5:27–33

Perez RH, Zendo T, Sonomoto K (2014) Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microb Cell Factories 13(1):3

Petyaev IM, Dovgalevsky PY, Chalyk NE et al (2014) Reduction in blood pressure and serum lipids by lycosome formulation of dark chocolate and lycopene in prehypertension. Food Sci NutrI 2(6):744–750

Praagman J, Dalmeijer GW, van der Schouw YT, Soedamah-Muthu SS et al (2015) The relationship between fermented food intake and mortality risk in the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort. British J Nutr 113(3):498–506

Pruska-Kędzior A, Kędzior Z, Gorący M, Pietrowska K et al (2008) Comparison of rheological, fermentative and baking properties of gluten-free dough formulations. Eur Food Res Technol 227(5):1523–1536

Quirós A, Hernández-Ledesma B, Ramos M et al (2005) Angiotensin-converting enzyme inhibitory activity of peptides derived from caprine kefir. J Dairy Sci 88(10):3480–3487

Rajaram G, Manivasagan P, Thilagavathi B, Saravanakumar A (2010) Purification and characterization of a bacteriocin produced by Lactobacillus lactis isolated from marine environment. Adv J Food Sci Technol 2(2):138–144

Ray B, Bhunia A (2008) Fundamental food microbiology, 4th edn. CRC Press, Boca Raton, pp 165–168

Ray RC, Panda SK, Swain MR et al (2012) Proximate composition and sensory evaluation of anthocyanin-rich purple sweet potato (Ipomoea batatas L.) wine. Int J Food Sci Technol 47(3):452–458

Rizzello CG, Nionelli L, Coda R et al (2010) Effect of sourdough fermentation on stabilisation, and chemical and nutritional characteristics of wheat germ. Food Chem 119(3):1079–1089

Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R et al (2010) Prebiotic effects: metabolic and health benefits. British J Nutr 104(2):1–63

Ross RP, Morgan S, Hill C (2002) Preservation an fermentation: past, present, future. Int J Food Microbiol 79:3–16

Saikali J, Picard C, Freitas M et al (2004) Fermented milks, probiotic cultures, and colon cancer. Nutr Cancer 49(1):14–24

Sanders ME (2009) How do we know when something called "probiotic" is really a probiotic? A guideline for consumers and health care professionals. Funct Food Rev 1:3–12

Sanders ME, Klaenhammer TR (2001) Invited review: the scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. J Dairy Sci 84:319–331

Sawada H, Furushiro M, Hiral K et al (1990) Purification and characterization of an anthlhypertensive compound from *Lactobacillus casei*. Agric Biol Chem 54:3211–3219

Slavin J (2013) Fiber and prebiotics: mechanisms and health benefits. Nutrition 5(4):1417–1435

Slavin JL, Lloyd B (2012) Health benefits of fruits and vegetables. Adv Nutr Int Rev J 3(4):506–516

Smith EE, Gui-Cheng H, John OI et al (2017) Some current applications, limitations and future perspectives of lactic acid bacteria as probiotics. Food Nutr Res 61:1318034

Solga SF (2003) Probiotics can treat hepatic encephalopathy. Med Hypotheses 61:307–313

Swain MK, Marimuthu A, Ray CR et al (2014) Fermented fruits and vegetables of Asia: a potential source of probiotics. Biotechnol Res Int 2014:250424

Tamang JP, Shin DH, Jung SJ et al (2016) Functional properties of microorganisms in fermented foods. Front Microbiol 7:578

Turroni F, Ventura M, Buttó LF, Duranti S et al (2014) Molecular dialogue between the human gut microbiota and the host: a Lactobacillus and Bifidobacterium perspective. Cell Mol Life Sci 71(2):183

Udenigwe CC, Aluko RE (2012) Food protein-derived bioactive peptides: production, processing, and potential health benefits. J Food Sci 77(1):11–24
Udhayashree N, Senbagam D, Senthilkumar B et al (2012) Production of bacteriocin and their application in food products. Asian Pac J Trop Biomed 2(1):406–410
Udompijitkul P, Paredes-Sabja D, Sarker MR (2012) Inhibitory effects of nisin against *Clostridium perfringens* food poisoning nonfood-borne isolates. J Food Sci 77(1):51–56
Veena V, Shriner KA et al (2010) Regulatory oversight and safety of probiotic use. Emerg Infect Dis 16(11):1661–1665
Vijayendra SVN, Halami PM (2015) Health benefits of fermented vegetable products. Health benefits of fermented foods and beverages. CRC Press/Taylor & Francis Group, Boca Raton, pp 325–342
Wu YY, Ding L, Xia HL et al (2010) Analysis of the major chemical compositions in Fuzhuan brick tea and its effect on activities of pancreatic enzymes in vitro. Afr Biotechnol 9(40):6748–6754
Yadav H, Jain S, Sinha PR (2007) Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats. Nutrition 23(1):62–68
Yadav R, Puniya AK, Shukla P (2016) Probiotic properties of *Lactobacillus plantarum* RYPR1 from an indigenous fermented beverage Raabadi. Front Microbiol 2(7):1683
Zhai H, Yang X, Li L et al (2012) Biogenic amines in commercial fish and fish products sold in southern China. Food Control 3:190–193
Zhang M, Peng C, Zhou Y (2000) Methods and compositions employing red rice fermentation products. US6046022O

Chapter 4
Advances in Fermentation Technology for Novel Food Products

Oluwafemi A. Adebo, Patrick B. Njobeh, Adedola S. Adeboye, Janet A. Adebiyi, Sunday S. Sobowale, Opeolu M. Ogundele, and Eugenie Kayitesi

Abstract The relevance of fermentation as an important and key aspect of food processing cannot be overemphasized, as it enhances beneficial composition and ensures safety. Fermentation technologies have constantly evolved with advances effectively dealing with the challenges associated with the traditional food fermentation process. Over the years, concerted efforts, intensive scientific research and the advent of modern sophisticated equipment have addressed these challenges and progressed to new approaches for fermentation of foods, subsequently leading to the delivery of novel food products. These advancements are further fueled by competitiveness among industry players based on innovativeness, cost-cutting measures, profit and the understandable desire for process improvement, better yields and quality products. This chapter covers significant advancement and technological applications that can improve food fermentation processes that are applicable for the delivery of better, safer and cost-effective food products.

Keywords Fermentation · Mixed cultures · Carbohydrate · Novel processing techniques · Food metabolomics · Nanotechnology

---

O. A. Adebo (✉) · P. B. Njobeh · J. A. Adebiyi · O. M. Ogundele · E. Kayitesi (✉)
Department of Biotechnology and Food Technology, University of Johannesburg, Doornfontein, Johannesburg, South Africa
e-mail: eugeniek@uj.ac.za

A. S. Adeboye
Department of Food Science, University of Pretoria, Hatfield, Pretoria, South Africa

Department of Food Technology, Moshood Abiola Polytechnic, Abeokuta, Ogun State, Nigeria

S. S. Sobowale
Department of Food Technology, Moshood Abiola Polytechnic, Abeokuta, Ogun State, Nigeria

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_4

Introduction

The onus and imperative for continued development of appropriate technologies for food production have continually risen over the past few years. While conventional food processing techniques still play an important role in the formulation of traditional diets, increasing consumer demand for high-quality, nutritious and safe products propels the industry to search for improved processes. Fermentation remains an age-long food processing technology, practiced, even before the understanding of the underlying processes involved. The techniques and associated knowledge involved in this process are normally handed down from one generation to the other and subsequently passed on within the local communities (Adebo et al. 2017a).

Recently, there has been an increased demand for fermented foods as potential sources of functional foods (Adebo et al. 2017a, b; Adebiyi et al. 2018). The need to meet consumer demand has made it essential to improve conventional fermentation techniques with advanced ones to ensure the delivery of desired fermented foods with consistently better quality, sensory attributes and nutritional benefits. This chapter, thus, provides an overview of the current state and potential developments and advances in fermentation technologies, for the delivery of novel food products. Aspects covered include the use of multi-strain starter cultures for fermentation, novel fermentation processes, carbohydrate for improved processes and other technological applications that can help enhance the development novel fermented foods.

Mixed Starter Cultures for Fermentation

Although most indigenous fermentation processes still largely rely on uncontrolled fermentation techniques (spontaneous fermentation and backslopping), the use of starter cultures (yeasts, bacteria and fungi) is desirable to ensure consistency, maintain hygiene, improve quality and guarantee constant sensory quality and composition. Sequel to the increased consumer demands for products with enhanced beneficial properties, the fermentation industry is constantly exploring ways to select, develop and use these starter cultures to improve the process. The general sequence used for the starter culture selection is depicted in Fig. 4.1. Commercial starter cultures are, however, not necessarily selected in this way but rather done based on rapid acidification and phage resistance (Leroy and De Vuyst 2004).

Starter cultures can be distinguished as single strain (one strain of a species), multi-strain (more than one strain of a single specie) or multi-strain mixed cultures (strains from different species) (Mäyrä-Mäkinen and Bigret 1998; Bader et al. 2010). While the use of single-strain cultures has been the norm and utilized for numerous food products, utilization of multi-strain and mixed cultures has demonstrated different advantages over single-strain use. Challenges of losses in the uniqueness, properties and characteristics of single-strain-fermented foods as

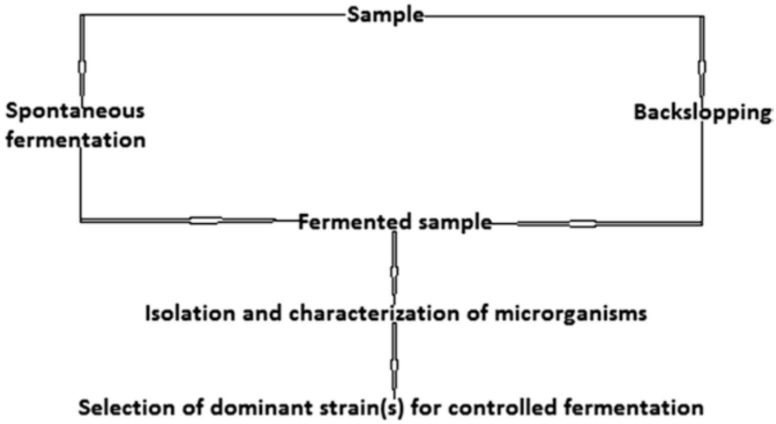


Fig. 4.1 Schematic diagram for strain selection

compared to different microorganisms have been reported (Caplice and Fitzgerald 1999), which could be ascribed to the limited microflora of the food. This could thus inform and suggest the use of multi-cultures, considering that these fermented foods are naturally produced through competitive action of different microorganisms and consequent varying metabolic pathways. Potential for synergistic utilization of different metabolic pathways; multiple biotransformation; increased yield; better organoleptic properties; bulk production of desirable metabolites, enzymes and antimicrobials; and rich biodiversity are added advantages of using mixed cultures (Meyer and Stahl 2003; Brenner et al. 2008; Bader et al. 2010).

Mixed cultures thus offer better complex metabolic activities and provide improved adaption in the food environment. Under such complex conditions, degradation, proteolysis, polymerization and metabolization of the inherent substrate occur through a combined metabolic activity of the inoculated strains. Examples of mixed culture applications for fermentation and delivery of novel food products are summarized in Table 4.1. Accordingly, through improved communication, trading of metabolites, exchange of molecular signals, combining tasks and division of labour among the cultures, better versatility and robustness are experienced under such conditions (Meyer and Stahl 2003; Brenner et al. 2008; Bader et al. 2010). The growth of one strain may however be enhanced or inhibited by the activities of another microorganism, and thus the production of primary and secondary metabolites may be increased or decreased (Keller and Surette 2006; Bader et al. 2010). Nonetheless, these cultures still play potential roles in increasing acidification and acceleration of the fermentation process and improvement of functionality, nutritional quality and health-promoting components.

Equally important are also reduction of cholesterol and biogenic amines and production of ɣ-aminobutyric acid (Ratanaburee et al. 2013; Kantachote et al. 2016) initiated through different interaction modes of mutualism, parasitism, competition, amensalism and commensalism between the strains. Through binding and production of metabolites, yeasts and LAB starter cultures have also been reported to

Table 4.1 Studies demonstrating the use of mixed cultures used for fermentation of food

Product	Raw material	Cocultures	Reference
Bread	Wheat flour, salt, sugar and water	*S. cerevisiae*, *Torulaspora delbrueckii* and *Pichia anómala*	Wahyono et al. (2016)
Cauim	Rice, cassava	*L. plantarum* and *Torulaspora delbrueckii*; *L. acidophilus* and *T. delbrueckii*	Freire et al. (2017)
Fermented milk	Milk	*C. kefyr* and *L. lactis*	Mufandaedza et al. (2006)
Fermented peanut milk	Peanut milk	*L. delbrueckii* ssp. *bulgaricus* *Streptococcus salivarius* ssp. *thermophilus*	Isanga and Zhang (2007)
Fermented sausage	Pork meat	*P. pentosaceus*, *L. sakei*, *S. xylosus*, *S. carnosus* and *Dabaryomyces hansenula*; *P. pentosaceus* and *S. xylosus*; *L. sakei* and *S. xylosus*	Wang et al. (2015)
Feta cheese	Pasteurized whole milk	*Lactococcus lactis*; *L. casei* and *Leuconostoc cremoris*; *L. lactis*, *L. casei* and *Enterococcus durans*; *L. lactis*, *L. casei*, *E. durans* and *Leuc. cremoris*	Litopoulou-Tzanetaki et al. (1993)
Functional beverage	Peanut-soy milk	*Saccharomyces cerevisiae* and *Pediococcus acidilactici*; *S. cerevisiae* and *Lactobacillus acidophilus*; *P. acidilactici* and *L. acidophilus*; *S. cerevisiae*, *P. acidilactici* and *L. acidophilus*	Santos et al. (2014)
Kefir	Milk	*Candida kefyr*, *Lactobacillus* sp., *Kluyveromyces* sp. and *Saccharomyces* sp.	Lopitz-Otsoa et al. (2006)
Moromi	Soy sauce	*Tetragenococcus halophilus* and *Zygosaccharomyces*; *T. halophilus* and *Z. rouxii*; *T. halophilus*, *Z. rouxii* and *Meyerozyma (Pichia) guilliermondii*	Singracha et al. (2017)
Nham	Pork	*P. pentosaceus* and *L. namurensis*	Ratanaburee et al. (2013) and Kantachote et al. (2016)
Probiotic beverage	Cereals	*L. plantarum* and *L. acidophilus*	Rathore et al. (2012)
Salami	Meat	*L. plantarum* and *L. curvatus*	Dicks et al. (2004), Todorov et al. (2007), and Bohme et al. (1996)
		L. sake and *Micrococcus* sp.; *L. curvatus* and *Micrococcus* sp.; *L. sake*, *L. curvatus* and *Micrococcus* sp.	
Suan yu	Fish	*L. plantarum*, *Stap. xylosus* and *S. cerevisiae*; *L. plantarum*, *Stap. xylosus* and *S. cerevisiae*; *P. pentosaceus*; *Stap. xylosus* and *S. cerevisiae*	Zheng et al. (2013)
Sucuk	Meat	*Staphylococcus carnosus* and *P. pentosaceus*; *Stap. carnosus* and *L. sakei*; *Stap. carnosus*, *P. entosaceus* and *L. sakei*	Bingol et al. (2014)

(continued)

Table 4.1 (continued)

Product	Raw material	Cocultures	Reference
Ting	Sorghum	*L. harbinensis* and *P. acidilactici*; *L. reuteri* and *L. fermentum*; *L. harbinensis* and *L. coryniformis*; *L. plantarum* and *L. parabuchneri*; *L. casei* and *L. plantarum*	Sekwati-Monang and Gänzle (2011)
Wine	Must	*S. cerevisiae* and *Starmerella bacillaris*	Tofalo et al. (2016)
Yakupa	Cassava	*S. cerevisiae* and *L. fermentum*; *T. delbrueckii* and *L. fermentum*; *P. caribbica* and *L. fermentum*	Freire et al. (2015)

detoxify mycotoxins (Adebo et al. 2017c). The use of cocultures/mixed starter cultures during the fermentation process would largely ensure a diversity of microflora that would provide a broad range of beneficial components in fermented foods. An in-depth understanding of the mechanisms of action of these multiple strains in a food system is however needed, necessitating further research in this regard.

Advances in the Use of Carbohydrates for Fermentation

The nature and type of carbohydrate influence inherent microbial and enzymatic actions as well as subsequent modifications to a substrate (Paulová et al. 2013). The complexity in the structural components of plant polysaccharides causes plant-degrading microbes to express numerous carbohydrate-active enzymes (CAZymes) (Lombard et al. 2014), which specifically modify or cleave to a specific type of sugar linkage (Boutard et al. 2014). Studies elucidating these mechanisms and approaches with models describing these interactions have been documented in the literature (Lynd et al. 2002; Boutard et al. 2014; Lombard et al. 2014; Lü et al. 2017).

Particularly, important advances on carbohydrates in fermentation technology are measures applied to improve enzyme accessibility to the active sites, thereby increasing digestibility of substrates during fermentation processes (Taherzadeh and Karimi 2008; Alvira et al. 2010; Lü et al. 2017). These pretreatments could be done using both chemical and physical methods. As for chemical methods, they include water pretreatment making a substrate suitable for enzymatic hydrolysis and subsequent fermentation as well as steam explosion because, high temperature is known to easily remove lignin, which might compromise microbial action (Taherzadeh and Karimi 2008; Thirmal and Dahman 2012). The major physical pretreatment commonly used is milling (Thirmal and Dahman 2012), with the assumption that it would physically increase the surface area of carbohydrates and improve accessibility of substrates to fermenting microbiota (Taherzadeh and Karimi 2008; Thirmal and Dahman 2012; Lü et al. 2017).

Equally important are non-digestible oligosaccharides (NDOs), which are low-molecular-weight carbohydrates, with intermediate properties between sugars and polysaccharides. Dietary fibre, an important member of this class, functions as prebiotics in diets, due to their excellent glycaemic response. Enrichment of fermenting substrates with NDOs gives an avenue for increasing bacterial population, biochemical profile and consequent beneficial physiological effects in the gut (Mussatto and Mancilha 2007). These NDOs have been produced from various carbohydrate sources via direct extraction from natural sources, chemical processes and hydrolyses of polysaccharides or by enzymatic action and chemical synthesis from disaccharides (Mussatto and Mancilha 2007). As such, NDOs are rapidly finding industrial applications both in prebiotic formulations and symbiotic products (containing probiotic organism and prebiotic oligosaccharide) (Mussatto and Mancilha 2007). This could potentially be utilized in different fermented foods for the delivery of desired health benefits.

Novel Food Processing Technologies for Improved Fermentation Processes

Novel and emerging food processing technologies for fermentation have increasingly gained interest over the past years. They are broadly categorized as a nonthermal and thermal process. The available novel nonthermal processes are high pressure processing (HPP), ultrasound (US) irradiation [gamma irradiation (ɣ-irradiation), microwave irradiation (MI)] and pulsed electric field (PEF); meanwhile thermal processes include ohmic heating (OH), radio frequency (RF) and microwave heating (MH). While the former could be aimed at accelerating the rate of chemical reactions (oxidation, polymerization, condensation and esterification) and fermentation, used for monitoring fermentation and for pasteurization, the latter may be adopted to improve shelf life, inactivate pathogenic and deleterious microorganisms, improve metabolic activities and production of enzymes as well as shorten the fermentation process. These techniques have recently been extensively described and adequately documented (Garde-Cerdán et al. 2016; George and Rastogi 2016; Koubaa et al. 2016a, b; Ojha et al. 2016, 2017). Available studies reporting the use of these technologies are summarized in Table 4.2.

HPP is conventionally applied to food products as a final mitigation step for products already packaged, which cannot be heat treated (Bajovic et al. 2012). They have received considerable attention as a technique for eliminating pathogens in fermented foods, although with mixed results. Some studies have indicated that HPP may not be desirable (Marcos et al. 2013; Omer et al. 2015), while others have encouraged the potential use of HPP in fermented foods (Table 4.2). Significant reduction and elimination in microbial loads of fermented foods have been reported (Omer et al. 2010; Gill and Ramaswamy 2008; Avila et al. 2016), with other studies indicating that HPP shortens wine ageing duration and enhances composition (Oey et al. 2008; Tchabo et al. 2017).

Table 4.2 Summary of studies reporting the use of novel processing techniques for fermented foods

Fermented product	Processing technique	Observation	Reference
Beer	US	Enhanced ethanol production	Choi et al. (2015)
Changran Jeotkal	ɣ-irradiation	Reduction of microbial levels, better chemical stability and improved overall acceptance	Jo et al. (2004)
Coffee	RF	Identification and characterization of behaviours during fermentation	Correa et al. (2014)
Dry-aged loins	US	Faster and better proteolytic changes in dry-aged meat cuts	Stadnik et al. (2014)
Fermented juice	OH	Retention of nutrients, inactivation of microorganisms	Profir and Vizireanu (2013)
Fermented milk	HPP	Reduced viable counts of *Candida* spoilage yeasts	Daryaei et al. (2010)
Fermented minced pepper	HPP	Lower levels of biogenic amines, lower microbial level, better sensory quality	Li et al. (2016)
Fermented sausage	ɣ-irradiation	Controlled the occurrence of undesirable and pathogenic microorganisms, reduction of *E. coli* O157:H7 load	Johnson et al. (2000), Chouliara et al. (2006), and Lim et al. (2008)
Fermented soybean paste	ɣ-irradiation	Reduction of biogenic amines	Kim et al. (2003)
Full-fat yoghurt	US	Higher water holding capacity, viscosity, lower syneresis and a reduction in fermentation	Hongyu et al. (2000)
Gochunjang	OH	Better pasteurization with no reduction in quality	Cho et al. (2016)
Kimchi	ɣ-irradiation	Controlled ageing and improved the shelf life of *kimchi*, sterilization of the product, softening of texture and better sensory quality	Song et al. (2004) and Park et al. (2008)
Kombucha analogues	PEF	Inactivation of acetic acid bacteria from *kombucha* consortium	Vazquez-Cabral et al. (2016)
Morr, *salami*	HPP	Reduction in *E. coli* O103:H25 and *E. coli* O157 counts	Gill and Ramaswamy (2008) and Omer et al. (2010)
Must	HPP	Reduction/elimination of wild microorganisms, especially yeasts	Bañuelos et al. (2016)
Must	MI	Reduction in fermentation time up to 40%, better alcohol yield	Kapcsándi et al. (2013)
Phellinus igniarius mycelial fermentation	US	Improved polysaccharides production, accelerated transfer of nutrients and metabolites	Zhang et al. (2014)

(continued)

Table 4.2 (continued)

Fermented product	Processing technique	Observation	Reference
Salami	HPP	Inactivation of *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* spp. and/or *T. spiralis* larvae	Proto-Fett et al. (2010)
Salted and fermented squid	ɣ-irradiation	Adequate squid fermentation, prevented putrefaction and prolonged shelf stability	Byun et al. (2000)
Seeds of *Plantago asiatica* L.	MH	Enhanced production of value-added polysaccharides	Hu et al. (2013)
Semihard cheese	HPP	Inactivation of *Clostridium tyrobutyricum* vegetative cells and prevention of late blowing defect	Avila et al. (2016)
Sugar cane must	ɣ-irradiation	Decrease in contaminating bacterial counts, decreasing acidity, improved ethanol yield	Alcarde et al. (2003)
Sweet whey	US	Reduced fermentation time, with higher viable counts	Barukcic et al. (2015)
Wine	ɣ-irradiation	Shortening of ageing time, improving rice wine defects, production of a higher taste quality	Chang (2003) and Chang (2004)
Wine	HPP	Increase in esters, aldehydes, ketones, terpenes, lactones and furans contents, reduction of fermentation time	Buzrul (2012) and Tchabo et al. (2017)
Wine	US	Shortened ageing time	Chang and Chen (2002), Chang (2004), and Liu et al. (2016)
Wine	PEF	Increased colour intensity, anthocyanins and total phenols, better extraction of bioactive compounds, higher flavonols and phenolics, reduction in the fermentation process time, alternative technique to stop fermentation (instead of using SO_2)	Lopez et al. (2008), Donsi et al. (2010), Puértolas et al. (2010), El Darra et al. (2013), Abca and Evrendilek (2015), Delsart et al. (2015), Mattar et al. (2015), and El Darra et al. (2016)
Wine	HVEF	Shortened wine maturation process	Zeng et al. (2008)
Wine	RF	Monitoring and quality control of traditional wine manufacturing	Song et al. (2015)
Yeast fermentation	US	Process signature which may be related to product and process quality was captured	Hoche et al. (2016)
Yoghurt	MH	Improved shelf life	Turgut (2016)
Yoghurt	US	Quality control and monitoring the fermentation stages of yoghurt	Alouache et al. (2015)

ɣ-irradiation gamma irradiation, *HPP* high pressure processing, *HVEF* high-voltage electric field, *MH* microwave heating, *OH* ohmic heating, *PEF* pulsed electric field, *RF* radio frequency, *US* ultrasound

Irradiating meat (with doses up to 3 kGy) prior to production of dry fermented pepperoni was reported to reduce microbial load of *E. coli* O157:H7, with resultant products possessing intact quality parameters (Johnson et al. 2000; Chouliara et al. 2006). Likewise is the use of microwave irradiation and heating, which have been applied in sterilization, material treatment and reduction in processing time, thus attracting a great deal of attention (Rasmussen et al. 2001; Hoai et al. 2011; Kapcsandi et al. 2013). The use of US also improves microorganism and/or enzyme activity, ensures high-quality product and safety (Alouache et al. 2015) and promotes esterification, oxidation and condensation reactions leading to the production of more esters, acids and esters in ageing processed wine (Tchabo et al. 2017) and milk (Nguyen et al. 2009, 2012).

The possibilities of future application and vast current use of electric current for fermentation and production of value-added products are promising (Cho et al. 2016). OH have been successfully applied in electroporation of microorganisms (Sastry 2005; Loghavi et al. 2008, 2009). In comparison with conventional heating, a decrease in lag fermentation phase with OH was demonstrated by Cho et al. (1996), suggesting it as a better technique for pasteurization and sterilization of viscous foods (Cho et al. 2016). Several applications of PEF in fermentation-related processes have been reported (Table 4.2), demonstrating improvement in the secretion of phenolic substances and anthocyanins (Puértolas et al. 2010), reduction of fermentation time, lesser browning and an improvement in yeast metabolism (Delsart et al. 2015; Mattar et al. 2015).

Limited studies have been presented on the use of RF in fermentation-related processes, with one of such observing increased homogeneity, retention of important microbes and no detrimental effect on storage stability of the yoghurt (Siefarth et al. 2014). Limitations of these novel technologies could relate to high investment costs, other variables during the process, standardization and optimization of the process to meet required regulations. Most of these applications reported are also under laboratory conditions, and simulating such under industrial conditions is needed to fully understand them and facilitate their subsequent implementation.

Other Techniques for Advancing and Improving Fermentation Processes

While other major technologies for the advancement of the fermentation process have been discussed, other potential technologies such as encapsulation, metabolomics and the use of extremophiles for the delivery of novel fermented food products are also highlighted in this section of the chapter.

Encapsulation for the Delivery of Novel Fermented Products

Encapsulation is a technique used to entrap active agents embedded in a carrier material to improve delivery of desired components into foods. It equally ensures protection of inherent materials (such as sensitive bioactive materials) against environmental extremes, stabilizes ingredients, immobilizes cells and enzymes during fermentation and can potentially mask unpleasant sensory qualities. Encapsulated starter cultures have demonstrated excellent applications in foods when compared to their nonencapsulated counterparts. They ensured stability and slow release of cultures during fermentation and production of heat-processed *sucuks* (Bilenler et al. 2017), higher viability during storage (Peredo et al. 2016) and an increase in fermentation efficiency and better microbial survival (De Prisco and Mauriello 2016; Simo et al. 2017).

Accordingly, encapsulation has been effectively used for the delivery of bioactive compounds and development of functional fermented foods. Increased folate-enriched functional foods was achieved using alginate and mannitol encapsulated LABs (Divya and Nampoothiri 2015), while a functional yoghurt was successfully produced by co-encapsulating bioactive compounds (Comunian et al. 2017). Bioactive compounds may also be nanoencapsulated such that their potential for use as antioxidants and antimicrobials is improved to ensure safety against opportunistic pathogenic microorganisms in fermented foods (Cushen et al. 2012). Nanoencapsulation has been applied to improve stability, protect nutraceuticals against degradation, enhance bioavailability and ensure the delivery of functional ingredients to potential consumers (Dasgupta et al. 2015).

Extremophiles for Fermentation

Extremophiles are microorganisms known to thrive in extreme conditions of pressure, pH, radiation, salinity and temperature, high levels of chemicals and osmotic barriers. Due to their ability to thrive under such conditions, they possess adaptive capabilities and contain enzymes with potential applications in diverse fields of biotechnology (Gomes and Steiner 2004; Adebo et al. 2017e). Extremozymes (enzymes from extremophiles) can effectively be applied to produce novel fermented foods mainly because they have naturally developed resistance to drastic changes and reactions during food processing. Examples of such extremozymes with potential applications include amylases, cellulases, proteases, catalases, xylanases, keratinases, pectinases, esterases, lipases, phytases and peroxidases (Gomes and Steiner 2004). Cold-active β-galactosidase has been utilized in the production of lactose free milk and cheese (Khan and Sathya 2017) and serine proteases applied for the hydrolysis of proteins to peptides (Mayr et al. 1996; de Carvalho 2011). Extremophilic lipases and esterasescan hydrolyze glycerols and fatty acids, with possibility of producing health promoting poly-unsaturated fatty acids in fermented foods (Schreck and Grunden 2014). Likewise are piezophilic extremozymes, which are also valuable to fermented food products requiring high-pressure processes (Zhang et al. 2015).

Food Metabolomics for the Delivery of Novel Food Products

Food metabolomics (foodomics) has facilitated the characterization and simultaneous determination of the comprehensive profile of foods (Adebo et al. 2017d). Qualitative and quantitative determinations of a complex food metabolome such as that of fermented foods, which had seemed technically challenging, can now be done sequel to the availability of sophisticated analytical equipment and chemometric tools. This profiling technique offers enormous potentials to generate in-depth information on the composition of fermented foods, metabolic interactions that can be associated with the functionalities and nutraceutical potentials embedded in fermented foods. Through the application of this technique, a thorough understanding of the effect of fermentation on the development of functional and novel fermented foods is feasible. Further to this is a better understanding of fermentation and how it influences product quality, functionality and desired properties.

Future Prospects and Conclusion

There is no doubt that fermentation is an integral and important processing technology employed in developing novel food products. Significant advances have been made over these past years on effective technologies needed for improving the fermentation processes. Different advanced technologies have emerged, and successful developments of novel food processing techniques and food products have equally been developed. The need, however, for improvement is inevitable with evolving food habits, consumer demand for better quality as well as stringent regulations. The use of the techniques highlighted in this chapter seems promising for modern industrial processes; nevertheless more detailed studies and optimization may still be required before they can be fully implemented on a large scale.

Acknowledgement We wish to acknowledge the financial support via the Global Excellence and Stature (GES) Fellowship of the University of Johannesburg (UJ) granted to the main author (Adebo, O.A.). This work was also partly supported by the National Research Foundation (NRF) Research and Technology Funding (RTF) and the National Equipment Programme (NEP) Grant.

References

Abca EE, Evrendilek GA (2015) Processing of red wine by pulsed electric fields with respect to quality parameters. J Food Process Preserv 39:758–767

Adebiyi JA, Obadina AO, Adebo OA, Kayitesi E (2018) Fermented and malted millet products in Africa: expedition from traditional/ethnic foods to industrial value added products. Crit Rev Food Sci Nutr 58:463−474

Adebo OA, Njobeh PB, Adebiyi JA, Gbashi S, Phoku JZ, Kayitesi E (2017a) Fermented pulse-based foods in developing nations as sources of functional foods. In: Hueda MC (ed) Functional food–improve health through adequate food. InTech, Croatia, pp 77–109

Adebo OA, Njobeh PB, Mulaba-Bafubiandi AF, Adebiyi JA, Desobgo ZSC, Kayitesi E (2017b) Optimization of fermentation conditions for *ting* production using response surface methodology. J Food Proc Preserv. In Press. 10.11/jfpp.13381

Adebo OA, Njobeh PB, Gbashi S, Nwinyi OC, Mavumengwana V (2017c) Review on microbial degradation of aflatoxins. Crit Rev Food Sci Nutr 57:3208–3217

Adebo OA, Njobeh PB, Adebiyi JA, Gbashi S, Phoku JZ, Kayitesi E (2017d) Food metabolomics: a new frontier in food analysis and its application to understanding fermented foods. In: Hueda MC (ed) Functional food–improve health through adequate food. InTech, Croatia, pp 211–234

Adebo OA, Njobeh PB, Sidu S, Adebiyi JA, Mavumengwana V (2017e) Aflatoxin B_1 degradation by culture and lysate of a *Pontibacter* specie. Food Cont 80:99–103

Alcarde AR, Walder JMM, Horii J (2003) Fermentation of irradiated sugarcane must. Sci Agric 60:677–681

Alouache B, Touat A, Boutkedjirt T, Bennamane A (2015) Monitoring of lactic fermentation process by ultrasonic technique. Phys Procedia 70:1057–1060

Alvira P, Tomás-Pejó E, Ballesteros M, Negro M (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour Technol 101:4851–4861

Avila M, Gomez-Torres N, Delgado D, Gaya P, Garde S (2016) Application of high pressure processing for controlling *Clostridium tyrobutyricum* and late blowing defect on semi-hard cheese. Food Microbiol 60:165–173

Bader J, Mast-Gerlach E, Popovic MK, Bajpal R, Stahl U (2010) Relevance of microbial coculture fermentations in biotechnology. J Appl Microbiol 109:371–387

Bajovic B, Bolumar T, Heinz V (2012) Quality considerations with high pressure processing of fresh and value added meat products. Meat Sci 92:280–289

Bañuelos MA, Loira I, Escott C, Del Frenzo JM, Morata A, Sanz PD et al (2016) Grape processing by high hydrostatic pressure: effect on use of non-saccharomyces in must fermentation. Food Bioprocess Technol 9:1769–1778

Barukcic I, Jakopovic KL, Herceg Z, Karlovic S, Bozanic R (2015) Influence of high intensity ultrasound on microbial reduction, physico-chemical characteristics and fermentation of sweet whey. Innov Food Sci Emerg Technol 27:94–101

Bilenler T, Karabulut I, Candogan K (2017) Effects of encapsulated starter cultures on microbial and physicochemical properties of traditionally produced and heat treated sausages (*sucuks*). LWT─Food Sci Technol 75:425–433

Bingol EB, Ciftcioglu G, Eker FY, Yardibi H, Yesil O, Bayrakal GM et al (2014) Effect of starter cultures combinations on lipolytic activity and ripening of dry fermented sausages. Ital J Anim Sci 13:776–781

Bohme HM, Mellet FD, Dicks LMT, Basson DS (1996) Production of salami from ostrich meat with strains of *Lactobacillus sake*, *Lactobacillus curvatus* and *Micrococcus* sp. Meat Sci 44:173–180

Boutard M, Cerisy T, Nogue PY, Alberti A, Weissenbach J, Salanoubat M et al (2014) Functional diversity of carbohydrate-active enzymes enabling a bacterium to ferment plant biomass. PLOS Genet 10:1–12

Brenner K, You L, Arnold FH (2008) Engineering microbial consortia: a new frontier in synthetic biology. Trends Biotechnol 26:483–489
Buzrul S (2012) High hydrostatic pressure treatment of beer and wine: a review. Innov Food Sci Emerg Technol 13:1–12
Byun MW, Lee KH, Kim DH, Kim JH, Yook HS, Ahn HJ (2000) Effects of gamma radiation on sensory qualities, microbiological and chemical properties of salted and fermented squid. J Food Protec 63:934–939
Caplice E, Fitzgerald GF (1999) Food fermentations: role of microorganisms in food production and preservation. Int J Food Microbiol 50:131–149
Chang AC (2003) The effects of gamma irradiation on rice wine maturation. Food Chem 83:323–327
Chang AC (2004) The effects of different accelerating techniques on maize wine maturation. Food Chem 86:61–68
Chang AC, Chen FC (2002) The application of 20 kHz ultrasonic waves to accelerate the aging of different wines. Food Chem 79:501–506
Cho HY, Yousef AE, Sastry SK (1996) Growth kinetics of *Lactobacillus acidophilus* under ohmic heating. Biotechnol Bioeng 49:334–340
Cho WI, Yi JY, Chung MS (2016) Pasteurization of fermented red pepper paste by ohmic heating. Innov Food Sci Emerg Technol 34:180–186
Choi EJ, Ahn H, Kim M, Han H, Kim WJ (2015) Effect of ultrasonication on fermentation kinetics of beer using six-row barley cultivated in Korea. J Inst Brew 121:510–517
Chouliara I, Samelis J, Kakouri A, Badeka A, Savvaidis IN, Riganakos K et al (2006) Effect of irradiation of frozen meat/fat trimmings on microbiological and physicochemical quality attributes of dry fermented sausages. Meat Sci 74:303–311
Comunian TA, Chaves IE, Thomazini M, Moraes ICF, Ferro-Furtado R, de Castro IA, Favaro-Trindade CS (2017) Development of functional yogurt containing free and encapsulated echium oil, phytosterol and sinapic acid. Food Chem 237:948–956
Correa EC, Jiménez-Ariza T, Díaz-Barcos V, Barreiro P, Diezma B, Oteros R et al (2014) Advanced characterisation of a coffee fermenting tank by multi-distributed wireless sensors: spatial interpolation and phase space graphs. Food Bioprocess Technol 7:3166–3174
Cushen M, Kerry J, Morris M, Cruz-Romero M, Cummins E (2012) Nanotechnologies in the food industry – recent developments, risks and regulation. Trends Food Sci Technol 24:30–46
Daryaei H, Coventry J, Versteeg C, Sherkat F (2010) Combined pH and high hydrostatic pressure effects on *Lactococcus* starter cultures and *Candida* spoilage yeasts in a fermented milk test system during cold storage. Food Microbiol 27:1051–1056
Dasgupta N, Ranjan S, Mundekkad D, Ramalingam C, Shanker R, Kumar A (2015) Nanotechnology in agro-food: from field to plate. Food Res Int 69:381–400
de Carvalho CCCR (2011) Enzymatic and whole cell catalysis: finding new strategies for old processes. Biotechnol Adv 29:75–83
De Prisco A, Mauriello G (2016) Probiotication of foods: a focus on microencapsulation tool. Trends Food Sci Technol 48:27–39
Delsart C, Grimi N, Boussetta N, Sertier CM, Ghidossi R, Peuchot MM et al (2015) Comparison of the effect of pulsed electric field or high voltage electrical discharge for the control of sweet white must fermentation process with the conventional addition of sulfur dioxide. Food Res Int 77:718–724
Dicks LMT, Mellet FD, Hoffman LC (2004) Use of bacteriocin-producing starter cultures of *Lactobacillus plantarum* and *Lactobacillus curvatus* in production of ostrich meat salami. Meat Sci 66:703–708
Divya JB, Nampoothiri KM (2015) Encapsulated *Lactococcus lactis* with enhanced gastrointestinal survival for the development of folate enriched functional foods. Bioresour Technol 188:226–230
Donsi F, Ferrari G, Fruilo M, Patara G (2010) Pulsed electric field-assisted vinification of Aglianico and Piedirosso grapes. J Agric Food Chem 58:11606–11165

El Darra N, Grimi N, Maroun RG, Louka N, Vorobiev E (2013) Pulsed electric field, ultrasound, and thermal pretreatments for better phenolic extraction during red fermentation. Eur Food Res Technol 236:47–56

El Darra N, Turk MF, Ducasse MA, Grimi N, Maroun RG, Louka N et al (2016) Changes in polyphenol profiles and color composition of freshly fermented model wine due to pulsed electric field, enzymes and thermovinification pretreatments. Food Chem 194:944–950

Freire AL, Ramos CL, Schwan RF (2015) Microbiological and chemical parameters during cassava based-substrate fermentation using potential starter cultures of lactic acid bacteria and yeast. Int J Food Microbiol 76:787–795

Freire AL, Ramos CL, de Costa Souza PN, Cardoso MGB, Schwan RF (2017) Nondairy beverage produced by controlled fermentation with potential probiotic starter cultures of lactic acid bacteria and yeast. Int J Food Microbiol 248:39–46

Garde-Cerdán M, Arias M, Martin-Belloso O, Acin-Azpilicueta C (2016) Pulsed electric field and fermentation. In: Ojha KS, Tiwari BK (eds) Novel food fermentation technologies. Springer, Switzerland, pp 85–123

George JM, Rastogi NK (2016) High pressure processing for food fermentation. In: Ojha KS, Tiwari BK (eds) Novel food fermentation technologies. Springer, Switzerland, pp 57–83

Gill AO, Ramaswamy HS (2008) Application of high pressure processing to kill *Escherichia coli* O157 in ready-to-eat meats. J Food Prot 71:2182–2189

Gomes J, Steiner W (2004) The biocatalytic potential of extremophiles and extremozymes. Food Technol Biotechnol 42:223–235

Hoai NT, Sasaki A, Sasaki M, Kaga H, Kakuchi T, Satoh T (2011) Synthesis, characterization, and lectin recognition of hyperbranched polysaccharide obtained from 1, 6-anhydro-D-hexofuranose. Biomacromolecules 12:1891–1899

Hoche S, Krause D, Hussein MA, Becker T (2016) Ultrasound-based, in-line monitoring of anaerobe yeast fermentation: model, sensor design and process application. Int J Food Sci Technol 51:710–719

Hongyu W, Hulbert GJ, Mount JR (2000) Effects of ultrasound on milk homogenization and fermentation with yogurt starter. Innov Food Sci Emerg Technol 1:211–218

Hu JL, Nie SP, LiC FZH, Xie MY (2013) Microbial short-chain fatty acid production and extracellular enzymes activities during in vitro fermentation of polysaccharides from the seeds of *Plantago asiatica* L. treated with microwave irradiation. JAgric Food Chem 61:6092–6101

Isanga J, Zhang GN (2007) Biologically active components and nutraceuticals in peanuts and related products: review. Food Rev Int 23:123–140

Jo C, Kim DH, Kim HY, Lee WD, Lee HK, Byun MW (2004) Studies on the development of low-salted, fermented, and seasoned *Changran Jeotkal* using the intestines of *Therage chalcogramma*. Radiation Phys Chem 71:121–124

Johnson SC, Sebranek JG, Olson DG, Wiegand BR (2000) Irradiation in contrast to thermal processing of pepperoni for control of pathogens: effects on quality indicators. J Food Sci 65:1260–1265

Kantachote D, Ratanaburee A, Sukhoom A, Sumpradit T, Asavaroungpipop N (2016) Use of ɣ-aminobutyric acid producing lactic acid bacteria as starters to reduce biogenic amines and cholesterol in Thai fermented pork sausage (*Nham*) and their distribution during fermentation. LWT-Food Sci Technol 70:171–177

Kapcsandi V, Nemenyi M, Lakatos E (2013) Effect of microwave treatment of the grape must fermentation process. In: food science conference Budapest, 2013–with research for the success Darenyi. Program 11:7–8

Keller L, Surette MG (2006) Communication in bacteria: an ecological and evolutionary perspective. Nat Rev Microbiol 4:249–258

Khan M, Sathya TA (2017) Extremozymes from metagenome: potential applications in food processing. Crit Rev Food Sci Nutr. In Press. https://doi.org/10.1080/10408398.2017.1296408

Kim JH, Ahn HJ, Kim DH, Jo C, Yook HS, Park HJ et al (2003) Irradiation effects on biogenic amines in Korean fermented soybean paste during fermentation. J Food Sci 68:80–84

Koubaa M, Barba-Orellana S, Rosello-Soto E, Barba FJ (2016a) Gamma irradiation and fermentation. In: Ojha KS, Tiwari BK (eds) Novel food fermentation technologies. Springer, Switzerland, pp 143–153

Koubaa M, Rosello-Soto E, Barba-Orellana S, Barba FJ (2016b) Novel thermal technologies and fermentation. In: Ojha KS, Tiwari BK (eds) Novel food fermentation technologies. Springer, Switzerland, pp 155–163

Leroy F, De Vuyst L (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol 15:67–78

Li J, Zhao F, Liu H, Li R, Wang Y, Liao X (2016) Fermented minced pepper by high pressure processing, high pressure processing with mild temperature and thermal pasteurization. Innov Food Sci Emerg Technol 36:34–41

Lim DG, Seol KH, Jeon HJ, Jo C, Lee M (2008) Application of electron-beam irradiation combined with antioxidants for fermented sausage and its quality characteristic. Radiation Phys Chem 77:818–824

Litopoulou-Tzanetaki E, Tzanetakis N, Vafopoulou-Mastrojiannaki A (1993) Effect of the type of lactic starter on microbiological, chemical and sensory characteristics of feta cheese. Food Microbiol 10:31–41

Liu L, Loira I, Morata A, Suarez-Lepe JA, Gonzalez MC, Rauhut D (2016) Shortening the ageing on lees process in wines by using ultrasound and microwave treatments both combined with stirring and abrasion techniques. Eur Food Res Technol 242:559–569

Loghavi L, Sastry SK, Yousef AE (2008) Effect of moderate electric field frequency on growth kinetics and metabolic activity of *Lactobacillus acidophilus*. Biotechnol Prog 24:148–153

Loghavi L, Sastry SK, Yousef AE (2009) Effects of moderate electric field frequency and growth stage on the cell membrane permeability of *Lactobacillus acidophilus*. Biotechnol Prog 25:85–94

Lombard V, Golaconda RH, Drula E, Coutinho PM, Henrissat B (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42:490–495

Lopez N, Puértolas E, Condón S, Álvarez I, Raso J (2008) Effects of pulsed electric fields on the extraction of phenolic compounds during the fermentation of must of Tempranillo grapes. Innov Food Sci Emerg Technol 9:477–482

Lopitz-Otsoa F, Rementeria A, Elquezabal N, Garaizar J (2006) Kefir: a symbiotic yeasts-bacteria community with alleged healthy capabilities. Rev lberoam Micol 23:67–74

Lü F, Chai L, Shao L, He P (2017) Precise pretreatment of lignocellulose: relating substrate modification with subsequent hydrolysis and fermentation to products and by-products. Biotechnol Biofuels 10:88

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS (2002) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev MMBR 66:506–577

Marcos B, Aymerich T, Garriga M, Arnau J (2013) Active packaging containing nisin and high pressure processing as post-processing listericidal treatments for convenience fermented sausages. Food Cont 30:323–330

Mattar JR, Turk MF, Nonus M, Lebovka NI, El Zakhem H, Vorobiev E (2015) *S. cerevisiae* fermentation activity after moderate pulsed electric field pre-treatments. Biochemist 103:92–97

Mayr J, Lupas A, Kellermann J, Eckerskorn C, Baumeister W, Peters J (1996) A hyperthermostable protease of the subtilisin family bound to the surface layer of the Archaeon *Staphylothermus marinus*. Curr Biol 6:739–749

Mäyrä-Mäkinen A, Bigret M (1998) Industrial use and production of lactic acid bacteria. In: Salminen S, von Wright A (eds) Lactic acid bacteria: microbiology and functional aspects. Marcel Dekker Inc, New York, pp 73–102

Meyer V, Stahl U (2003) The influence of co-cultivation on expression of the antifungal protein in *Aspergillus giganteus*. J Basic Microbiol 43:68–74

Mufandaedza J, Viljoen BC, Feresu SB, Gadaga TH (2006) Antimicrobial properties of lactic acid bacteria and yeast-LAB cultures isolated from traditional fermented milk against pathogenic *Escherichia coli* and *Salmonella enteritidis* strains. Int J Food Microbiol 108:147–152

Mussatto SI, Mancilha IM (2007) Non-digestible oligosaccharides: a review. Carbohydr Polym 68:587–597

Nguyen TMP, Lee YK, Zhou W (2009) Stimulating fermentative activities of Bifidobacteria in milk by high intensity ultrasound. Int Dairy J19:410–416

Nguyen TMP, Lee YK, Zhou W (2012) Effect of high intensity ultrasound on carbohydrate metabolism of Bifidobacteria in milk fermentation. Food Chem 130:866–874

Oey I, Lille M, Van Loey A, Hendrickx M (2008) Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. Trends Food Sci Technol 19:320–328

Ojha KS, O'Donnell CP, Kerry JP, Tiwari BK (2016) Ultrasound and food fermentation. In: Ojha KS, Tiwari BK (eds) Novel food fermentation technologies. Springer, Switzerland, pp 125–142

Ojha KS, Mason TJ, O'Donnell CP, Kerry JP, Tiwari BK (2017) Ultrasound technology for food fermentation applications. Ultrason Sonochem 34:410–417

Omer MK, Alvseike O, Holck A, Axelsson L, Prieto M, Skjerve E, Heir E (2010) Application of high pressure processing to reduce verotoxigenic *E. coli* in two types of dry-fermented sausage. Meat Sci 86:1005–1009

Omer MK, Prieto B, Rendueles E, Alvarez-Ordonez A, Lunde K, Alvseike O, Prieto M (2015) Microbiological, physicochemical and sensory parameters of dry fermented sausages manufactured with high hydrostatic pressure processed raw meat. Meat Sci 108:115–119

Park JG, Kim JH, Park JN, Kim YD, Kim WG, Lee JW et al (2008) The effect of irradiation temperature on the quality improvement of *Kimchi*, Korean fermented vegetables, for its shelf stability. Radiat Phys Chem 77:497–502

Paulová L, Patáková P, Brányik T (2013) Engineering aspects of food biotechnology. CRC Press, Boca Raton, pp 89–110

Peredo AG, Beristain CI, Pascual LA, Azuara E, Jimenez M (2016) The effect of prebiotics on the viability of encapsulated probiotic bacteria. LWT─Food Sci Technol 73:191–196

Profir A, Vizireanu C (2013) Effect of the preservation processes on the storage stability of juice made from carrot, celery and beetroot. J Agroaliment Proc Technol 19:99–104

Proto-Fett ACS, Call JE, Shoyer BE, Hill DE, Pshebniski C, Cocoma GJ et al (2010) Evaluation of fermentation, drying, and/or high pressure processing on viability of *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* spp., and *Trichinella spiralis* in raw pork and Genoa salami. Int J Food Microbiol 140:61–75

Puértolas E, López N, Saldaña G, Álvarez I, Raso J (2010) Evaluation of phenolic extraction during fermentation of red grapes treated by a continuous pulsed electric fields process at pilot-plant scale. J Food Eng 98:120–125

Rasmussen MJ, Rea RF, Tri JL, Larson TR, Hayes DL (2001) Use of a transurethral microwave thermotherapeutic device with permanent pacemakers and implantable defibrillators. Mayo Clin Proc 76:601–603

Ratanaburee A, Kantachote D, Charernjiratrakul W, Sukhoom A (2013) Enhancement of ɣ-aminobutyric acid (GABA) in *Nham* (Thai fermented pork sausage) using starter cultures of *Lactobacillus namurensis* NH2 and *Pediococcus pentosaceus* HN8. Int J Food Microbiol 167:170–176

Rathore S, Salmeron I, Pandiella S (2012) Production of potentially probiotic beverages using single and mixed cereal substrates fermented with lactic acid bacteria cultures. Food Microbiol 30:239–244

Santos CC, Libeck-Bda S, Schwan RF (2014) Co-culture fermentation of peanut-soy milk for the development of a novel functional beverage. Int J Food Microbiol 186:32–41

Sastry SK (2005) Advances in ohmic heating and moderate electric field (MEF) processing. In: Barbosa-Canovas GV, Tapia MS, Cano MP (eds) Novel food processing technologies. CRC Press, Boca Raton

Schreck SD, Grunden AM (2014) Biotechnological applications of halophilic lipases and thioesterases. Appl Microbiol Biotechnol 98:1011–1021

Sekwati-Monang B, Gänzle MG (2011) Microbiological and chemical characterisation of *ting*, a sorghum-based sourdough product from Botswana. Int J Food Microbiol 150:115–121

Siefarth C, Bich T, Tran T, Mittermaier P, Pfeiffer T, Buettner A (2014) Effect of radio frequency heating on yoghurt, I: technological applicability, shelf-life and sensorial quality. Foods 3:318–335

Simo G, Vila-Crespo J, Fernández-Fernández E, Ruipérez V, Rodríguez-Nogales JM (2017) Highly efficient malolactic fermentation of red wine using encapsulated bacteria in a robust biocomposite of silica-alginate. J Agric Food Chem 65:5188–5197

Singracha P, Niamsiri N, Visessanguan W, Lertsiri S, Assavanig A (2017) Application of lactic acid bacteria and yeasts as starter cultures for reduced-salt soy sauce (*moromi*) fermentation. LWT–Food Sci Technol 78:181–188

Song HP, Kim DH, Yook HS, Kim KS, Kwon JH, Byun MW (2004) Application of gamma irradiation for aging control and improvement of shelf-life of *kimchi*, Korean salted and fermented vegetables. Radiat Phys Chem 71:55–58

Song H, Choi J, Park CW, Shin DB, Kang SS, Oh SH, Hwang K (2015) Study of quality control of traditional wine using it sensing technology. J Korean Soc Food Sci Nutr 44:904–911

Stadnik J, Stasiak DM, Dolatowski ZJ (2014) Proteolysis in dry-aged loins manufactured with sonicated pork and inoculated with *Lactobacillus casei* ŁOCK 0900 probiotic strain. Int J Food Sci Tech 49:2578–2584

Taherzadeh MJ, Karimi K (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int J Mol Sci 9:1621–1651

Tchabo W, MaY KE, Zhang H, Xiao L, Tahir HE (2017) Aroma profile and sensory characteristics of a sulfur dioxide-free mulberry (*Morus nigra*) wine subjected to non-thermal accelerating aging techniques. Food Chem 232:89–97

Thirmal C, Dahman Y (2012) Comparisons of existing pretreatment, saccharification, and fermentation processes for butanol production from agricultural residues. Can J Chem Eng 90:745–761

Todorov SD, Koep KSC, Van Reenen CA, Hoffman LC, Slinde E, Dicks LMT (2007) Production of salami from beef, horse, mutton, Blesbok (*Damaliscus dorcas phillipsi*) and Springbok (*Antidorcas marsupialis*) with bacteriocinogenic strains of *Lactobacillus plantarum* and *Lactobacillus curvatus*. Meat Sci 77:405–412

Tofalo R, Patrignani F, Lanciotti R, Perpetuini G, Schirone M, Gianvito D et al (2016) Aroma profile of Montepulciano d'Abruzzo wine fermented by single and co-culture starters of autochthonous *Saccharomyces* and non-*Saccharomyces* yeasts. Front Microbiol 7:1–12

Turgut T (2016) The effect of microwave heating on some quality properties and shelf life of yoghurt. Kafkas Univ Vet Fak Derg 22:809–814

Vazquez-Cabral D, Valdez-Fragoso A, Rocha-Guzman NE, Moreno-Jimenez MR, Gonzalez-Laredo RF, Morales-Martinez PS et al (2016) Effect of pulsed electric field (PEF)-treated *kombucha* analogues from *Quercus obtusata* infusions on bioactives and microorganisms. Innov Food Sci Emerg Technol 34:171–179

Wahyono A, Lee SB, Kang WW, Park HD (2016) Improving bread quality using co-cultures of *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* JK08, and *Pichia anomala* JK04. Ital J Food Sci 28:298–313

Wang X, Ren H, Wang W, Zhang Y, Bai T, Li J et al (2015) Effects of inoculation of commercial starter cultures on the quality and histamine accumulation in fermented sausages. J Food Sci 80:377–383

Zeng AA, Yu SJ, Zhang L, Chen XD (2008) The effects of AC electric field on wine maturation. Innov Food Sci Emerg Tech 9:463–468

Zhang H, Ma H, Liu W, Pei J, Wang Z, Zhou H, Yan J (2014) Ultrasound enhanced production and antioxidant activity of polysaccharides from mycelial fermentation of *Phellinus igniarius*. Carbohydr Polym 113:380–387

Zhang Y, Li X, Bartlett DH, Xiao X (2015) Current developments in marine microbiology: high-pressure biotechnology and the genetic engineering of piezophiles. Curr Opin Biotechnol 33:157–164

Zheng X, Xia W, Jiang Q, Yang F (2013) Effect of autochthonous starter cultures on microbiological and physico-chemical characteristics of *Suan yu*, a traditional Chinese low salt fermented fish. Food Cont 33:344–351

Chapter 5
Advances in Technology and New Product Development in the Beer, Wine, and Spirit Industry

Inge Russell and Julie Kellershohn

Abstract Food and beverage trends in 2017 can be attributed mainly to the rise and impact of the spending power and consumer demands of the millennial generation. With the desire for authenticity and locally sourced products, there is a rapid rise in craft distilling along with craft breweries that also distill (the brewstillery). Hybrid alcoholic products are eliminating the barriers between traditional beverage categories. Fermented products such as baijiu are moving into new geographic regions. Desire for convenience is seen in the rise of single-serve packaging and the myriad of new and innovative wine and spirit packaging options. Health consciousness is reflected in the increased sales of lower alcohol and no alcohol beers. Products such as edible beer six pack rings address consumer concern for the environment. Quality, product integrity, transparency, traceability, and labeling of nutritional and calorie content are some of the keys to capturing the millennial market.

Keywords Alcoholic beverages · Beer · Craft · Innovation · Low alcohol · Marketing · Packaging · Spirits · Wine

Introduction

Although the production of alcoholic beverages is an ancient craft, utilizing techniques developed and refined over a few millennia, it is also an industry where small process innovations are still being developed, which ensure consistent product quality and enhance the economics of production. Trends in 2017 can be attributed

I. Russell (✉)
Russell & Associates, London, ON, Canada

J. Kellershohn
Ryerson University, Toronto, ON, Canada

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_5

mainly to the rise and impact of the spending power and consumer demands of the millennial generation. Consumers value health consciousness, the environment, and convenience, and these trends translate into a movement toward more single-serve packaging innovations, lighter alcohol beverages, premiumization, traceability, and transparency throughout the entire manufacturing process, from raw materials until delivery to the consumer. There is a rapid rise in craft distilling, similar to what was seen in craft brewing a number of years ago (and is still occurring). There is growing interest in the home production of fermented beverages, using various types of innovative equipment designed for easy home use and accessibly priced for the home brewer.

Hybrid Products and Unusual Combinations

The rise in hybrid drinks is eliminating barriers between traditional categories, leading to category blur among beer, wine, and spirits. These drinks are a blend of ingredients from multiple beverage categories with new category names such as speers (spirit beers) and spiders (spirit ciders).

The premixed cocktail sector, which has traditionally been present in the spirit sector, is finding new traction. It is becoming challenging to differentiate among premixed cocktails and to identify if the base of the cocktail in the retail outlet is a beer, cider, wine, or spirit. Single portion servings in this category invite consumers to try the wide variety of products that now dominate the shelves. The rapid expansion in product offerings has made it harder for brands to differentiate themselves unless they leverage innovative packaging, social media, or nontraditional concepts to help them stand out from the pack.

For example, traditional drinks, such as Scotch whisky, have expanded their consumer base by introducing canned cocktail ready-to-drink (RTD) product extensions. The Scottish Finnieston Distillery Company, which won the World Beverage Innovation Awards 2017 in the "best premium or adult drink category," offers a range of RTD canned cocktails made with Scottish whisky (e.g., one canned cocktail's ingredients are Scotch whisky, ginger beer, vanilla, and strawberry). The producing company states "We take the view that people should drink it [Scotch] how they want, not how they are instructed."

Alcohol-Free and Low-Alcohol Beers

The increasing popularity of this growing market segment reflects the millennial generation's consumer interest in healthier lifestyle choices, which includes products with lower alcohol and lower sugar levels. The megabrands have identified that there is high growth potential in this market. AB InBev has stated that its goal is to have lower-alcohol beers or alcohol-free beers comprise at least 20% of their global beer volume by 2025. These companies are also investigating the massive markets of China, Brazil, Argentina, and Mexico, where there are large consumer groups who abstain from alcohol, and there is an opportunity to target these growing consumer groups with innovative lower- or no-alcohol products.

Some low-alcohol beers are now being marketed by producers as an alternative to soft drinks, as consumers move away from highly sweetened sodas. In countries such as Germany, these low-alcohol beers are positioned as exercise drinks with taglines such as "a refreshing isotonic recovery drink."

Alcohol-free and low-alcohol beers in the past have suffered from a variety of defects (e.g., sweet and cloying flavors, yeasty tastes, lack of proper mouthfeel). These flavor problems, when trying to match a mainstream beer, are slowly being solved in creative ways, and one can expect in the future to see much better flavored low-alcohol beers enter the marketplace, as modern technologies are invoked and esters and other flavor profiles are better understood and managed.

According to the Mintel Global New Products Database (GNPD), China is currently the leader in terms of launching innovative low- or no-alcohol products. In 2016, over one in four beers launched in China (29%) were listed as low alcohol (under 3.5% ABV) or no alcohol. This contrasts with lower launch figures in Spain (12%), Germany (11%), Poland (9%), and the UK (7%).

Each country defines its own legal regulations for what is considered to be an alcohol-free product vs a low-alcohol product, but many use the guideline that "no-alcohol" products contain no more than 0.05% alcohol and that "lower-alcohol" products contain no more than 1.2% alcohol.

There are several ways to produce an alcohol-free beer. The alcohol can be removed after the beer is brewed, or the fermentation can be stopped before the alcohol is produced but after flavor compounds have been produced, or some combination thereof. Of the various technologies that can be used to remove the alcohol content, the simplest is the use of heat to remove the alcohol, but this can negatively affect beer flavor (many delicate flavor compounds suffer as a result of heat treatment). Vacuum distillation, to remove the alcohol, results in a much better final product because lower heating temperatures are employed. Reverse osmosis is an even gentler technology to remove alcohol, as this is a technology that does not employ heat treatment, but it is a slower and more labor-intensive process.

Alfa Laval has pioneered a fully automated de-alcoholization module, which reduces the alcohol level to under 0.05% alcohol, at both low temperatures and pressures with just a single pass of the product through a vertical stripping column. This approach uses fewer utilities by using a combination of heat exchangers. The module first degasses the beer, returns the condensable volatiles into the beer stream, and removes the alcohol using culinary steam flowing upward, in near vacuum, in a special stripping column. The alcohol, which exits as a vapor from the column, is condensed, collected, and can then be used for other purposes.

The Rise in Craft Distilling

Millennial consumers have a strong affinity for local “farm-to-glass” products. Craft spirits, similar to craft beers, tend to sell in the premium price category, touting authenticity, craftsmanship, and the concept of “drinking less but better.” There are currently over 1300 craft distilleries in the USA, and spirit sales have grown from 2010 to 2015 at an astounding rate of 28% annually. The American Craft Spirits Association (www.americancraftspirits.org) reports that the craft market is now at 3% of the spirit market share. Marketing via social media, as was seen with craft beers, is one of the main drivers of the products’ success.

Craft Breweries That Also Distill (the Brewstillery)

Why are there currently many craft breweries that also distill? The answer is simple. The brewery already contains all of the equipment needed for the front end of the distilling process: producing the mash for fermentation. To the end of fermentation, the process is similar. The main difference being that a distiller’s wort is not hopped and not boiled. Some brewers and distillers partner on site, with a distillery built next to a brewery. Others build the distillery further away from the brewery (often due to local regulations regarding the building of a distillery) and transport the partially finished beverage from the brewery to a second location, where the distillation and aging steps can be carried out.

Distilleries are being established in large numbers as the laws prohibiting distillation are being relaxed or are evolving to be more flexible. With the current market demand, equipment options for small distilleries have increased in number and decreased in both price and size to accommodate the considerable number of start-up craft distillers. The selection of available distillation equipment from micro to large distillery has never been greater. All-in-one systems that can be designed to configure any type of still and permit the production of more than one style of spirit are very popular.

Rise in Home Craft Brewing and Distilling

Pico is a US company that offers several countertop beer machines from the starter option, the “PicoBrew,” to the more advanced larger “Zymatic” countertop beer maker. These beer machines were developed with the vision of simplifying the brewing process for home brewers. The “Zymatic” is web-connected and provides temperature data at each step of the process and transfers the wort when ready – a “set it and forget it program” – so that a novice brewer can make an acceptable batch of beer every time with mechanization and standardization being their keys to success. The Zymatic machine, priced at close to $2,000USD, while not inexpensive is priced similar to a high-end cappuccino machine. Other home brewing machines that are popular include the New Zealand "Grainfather" and the German "Braumeister."

There is great interest in craft home distilling as evidenced by the PicoStill – a Kickstarter campaign by Pico, which raised more than $800,000 USD in just 2 weeks and, to date, is one of the top 10 most successful food Kickstarter campaigns of all time! The PicoStill is a distilling attachment that is compatible with the company's brewing technology described above. It can be used to distill hop oil, water, essential oils, and spirits, although depending on the location, proper licenses and permits may be needed for the spirits.

Keurig-Type Brewing and Distilling Systems

There are a number of machines being developed similar to the concept of a Keurig coffee machine for making beer at home. In 2016, a leading sparkling water maker (SodaStream) launched a homemade beer system in Germany and Switzerland (The Beer Bar), which uses sparkling water and a beer concentrate and, when mixed, the final product is 4.5% alcohol. A US company (Pat's Backcountry Beverages) has partnered the use of its concentrate with a sparkling water company (Sparkling Drink Systems) to produce a homemade beer product. In addition, there is a product sold for campers who do not want to carry heavy bottles of beer. A patented beer concentrate is produced by what the inventors at Pat's Backcountry Beverages describe as "a unique labor-intensive process." This concentrate can be mixed with springwater, while on outdoor adventures, to produce a final product on which consumer feedback has been reasonably positive.

Is It Possible to Reduce Spirit Aging Time with Technology?

The lure of innovative technology that could rapidly age spirits such as whisky and rum has long been an elusive goal, but there are a number of companies actively pursuing the concept using a variety of different approaches. If the product taste resulting from years of aging could be duplicated, the benefit to the producer of being able to rapidly bring the product to market and save the expense of aging warehouses is an attractive economic proposition.

There are a number of US companies actively engaged in exploring the burgeoning field of rapid aging technologies. Terressentia Corporation in South Carolina, with their TerrePURE system (a combination of ultrasonic energy and oxygenation), has developed technology with the goal of reducing unwanted harsh-tasting congeners, and transforming harsh-tasting acids into pleasant tasting esters through a rapid aging process.

Cleveland Whiskey in Ohio (using a process which includes temperature and pressure cycles) has products that have won gold medals in industry competitions, and they were named Whiskey Innovator of the Year at the 2016 Berlin International Spirits Competition. Their patented pressure-aging technology appears to be producing very drinkable innovative products for the marketplace.

In Spain, researchers studying brandy have obtained promising results when they funnel the brandy through a glass tube and a bed of American oak chips for 3 days while at the same time subjecting the brandy to ultrasound. They reported that they had produced a pleasing product, not identical but close to a brandy aged for 2 years. It is speculated that the ultrasound treatment induces cavitation in the wood chips, releasing the congeners present in the wood tissue into the spirit (Delgado-González et al. 2017).

Lost Spirits in California has a process originally developed for rum by Bryan Davis, which includes what they refer to as **t**argeted **h**yper-**e**sterification **a**ging (THEA) (a heat-driven esterification and photocatalytic polymer degradation of oak barrel staves). In 2017, Lost Spirits launched two products that used new-make spirit from Scotland and their rapid aging THEA technology with the use of American oak staves (toasted/charred). The products were priced at a premium level and were named after chapters from H. G. Wells's *The Island of Doctor Moreau* – Abomination "The Crying of the Puma" and Abomination "The Sayers of the Law." The designation "whisky" is not used on the bottles, merely the wording "spirits distilled from 100% malted barley finished with late harvest Riesling seasoned American oak staves."

Innovators, due to labeling legislation in their respective countries, in many cases, will not be allowed to call a rapidly aged product a brandy or a whisky when commercialized but rather will have to market the product as a different offering, such as the previously mentioned Lost Spirits.

The various rapid aging technologies described above, although not all yet perfected to create perfect product matches, are all very useful for research and product development. For example, experimenting with different woods could give insights into possible flavor outcomes using novel woods, not normally a part of the manufacturer's traditional process, without having to wait many years for tastings of the experimental products. Product aging acceleration can be expected to be a technology that will continue to be of great interest to the industry and that will lead to many future innovative products.

How Consumer Preference Is Changing Wine Packaging

Wine in Cans: The Market

The wine industry in the past has not been a leader in packaging innovation, for the most part, relying on traditional glass bottles. In the past few years, this has been changing (Forbes 2016), with the expansion of packaging wines into cans. In 2017, there has been a significant boom in the sale of wine in cans, with some stores struggling to maintain the product on the shelf. Wine in cans addresses the consumer desire for the option of a single-serving product and a package that is also very portable. Millennials, particularly women, were the first market that embraced this concept of lighter wines meant to be drunk cold and packaged in single-serve cans. The familiarity and acceptance of the presence of high-end artisanal beers in cans have helped to make wine in cans more acceptable as a packaging alternative to millennial consumers. Some marketing campaigns go so far as to leverage the anti-snob vibe that a can gives, leveraging this into how they promote their wines in cans.

Wine in Cans: The Technology

Although bottle manufacturers have developed glass that is lighter and still strong enough to withstand the impact of carbon dioxide that comes from a secondary fermentation, there are many markets where glass is not an option, as bottles are banned or are impractical. Aluminum cans have a very high global recycling rate compared to glass and PET. Almost 17 times lighter than standard bottles, cans offer improved shipping efficiency and less waste due to breakage.

In cans, the wine is well protected from oxygen, which is, over time, the main villain in terms of storability. While protection from oxygen is ideal for wines that have a bright and fresh profile, for some wines this complete exclusion of oxygen can pose a problem where the flavor profile includes the effects of minuscule amounts of oxygen which, over years, enter the wine through the cork.

Metallic aftertaste is no longer a concern with wines in cans due to modern linings. An epoxy resin coating (BPA-free) inside of the can ensures that the aluminum does not touch the beverage.

Crushability of cans is a concern when there is a desire for thin light cans to reduce their carbon footprint. Carbonated beverages are easier to accommodate in this respect as the carbon dioxide pressure inside of the can, as with canned beer, gives it the ability to withstand crushing. For non-sparkling wine beverages, liquid nitrogen can be used. When injected into the can, the liquid nitrogen becomes a gas as it warms, and in its gas form, the nitrogen occupies more space than the liquid form, pressing against the walls of the can to give it enhanced non-crushability.

Wine in Tetra Pak

Wine in Tetra Pak is not a new type of packaging, but it is growing in market share. Consumers want more options in terms of package size from single servings to large resealable cartons. The technology of the carton involves three materials, paperboard, polyethylene, and aluminum. These three materials are compressed using heat and pressure to form a six-layer protection for the product, and the resultant packaging is able to keep out light, air, and moisture. The main negative to this packaging is that it does not send a marketing message of a high-end wine even though the product inside could very well be very high-end.

Wine-in-a-Box

Wine packaged in bag-in-box is not a new concept but is increasing in popularity because of its utilitarian approach to storage and ease of use. The larger packaging size leads to lower shipping costs, which means the wines can be offered at a lower price point, a clear benefit in the mind of the consumer. The bag-in-the-box concept uses a sophisticated system of multilayers of different extruded plastics. The sealed spout does not allow air into the package, and by stopping oxygen contact with the wine, it extends freshness to 4 (or even up to 6) weeks after the first glass is poured. While some wine consumers look down on this type of packaging, some marketing companies leverage this fact by turning the tables, mocking wine connoisseur's pretentions, and stressing that it is about the taste of the wine in the glass and not the supposed status that the packaging confers.

Attitudes about bag-in-box wines in the past 2 years have changed, and it is now considered a "smart casual" product purchase. Although at one time bag-in-box wines were not usually award-winning vintages, this has also recently changed. The popularity of this type of packaging with the eco-conscious consumer has driven demand for higher-quality wines, and a number of wineries are now delivering some of their top products using this packaging.

Wine on Tap in Restaurant Kegs

Addressing many of the same issues that wine packaged in cans addresses (single servings and offering the ability to try multiple different glasses without committing to an entire bottle), wine in kegs in restaurants is finding a growing market. As with beer, there is the use of stainless steel and PET kegs, with PET finding growth since there is no need to keep a return and refill system in place, thus lowering costs when shipping long distances.

A main barrier to entry in the past was the lack of a structured system for keg filling, return, and refill, similar to what has already been in place in the beer industry for many years. Wine kegs require type 304 stainless steel and special hosing to prevent oxygen ingress (oxygen barrier tubing). Type 303 stainless steel cannot be used as the brass corrodes under the pH of the wine resulting in sulfur and taint flavors. The gas that pushes the wine out to the tap is a blend of gases (such as 75% nitrogen and 25% CO_2).

Wine-on-tap in PET kegs is a popular option, since one 20 L PET keg replaces 27 glass bottles and eliminates waste, spoilage due to cork taint, and breakage. The brown keg color can protect the wine from UV light, and companies incorporate their own oxygen barrier technologies to restrict oxygen ingress and reduce CO_2 loss. Sparging the keg with nitrogen before filling and using nitrogen to remove oxygen from the headspace of the keg protects the wine. For example, an untapped petainerKeg has a 9–12-month shelf life and a 2-month shelf life once tapped. PET kegs offer the restaurant the opportunity to present a greater selection of wines as well as allowing them to serve it in a variety of glass sizes, making it easier to offer customers small tastes, enhancing the wine selection experience for them.

Wine in Technology-Enhanced Packaging

Wine is also starting to appear in technology-enhanced packaging such as the Kuvée system. Here, technology innovation, in terms of innovative packaging, pairs with product information technology. The Kuvée system involves a system that allows you to swap wine canisters in and out of it, and after pouring from the system, it seals the canister, keeping it fresh and allowing for multiple canisters to be open at once, with each staying fresh for up to 30 days. The container into which the canister is inserted lights up a screen on the front, with the wine's label and backstory, suggestions for food pairings, and information on how much wine is left in the bottle becomes visible to the consumer. There is also the ability for the consumer to order refills (directly from the software embedded in the bottles) and to have the bottles auto shipped via the Kuvée e-store.

Wine in Zipz Recyclable Glasses

Single-serve recyclable containers in the shape of a wine glass are another example of new packaging technology by Zipz. Their single-serve resealable glasses consist of eco-friendly PET and a patented wrapping technology to keep oxygen out and to prevent spillage, until the glass is unzipped from its covering. Once unzipped, the lid then forms the base of the glass. This PET wine glass won the 2015 Beverage World Functionality Award for its convenience and ability to be taken into venues where glass is not an option.

Wine Dispensing Systems

Attractive large systems that hold multiple bottles of wine with touch screen capability, temperature, and volume control (e.g., the Enomatic system can hold 16 wine bottles in one unit) and allow the wine to be drawn directly from a bottle to the glass through a gas system (nitrogen or argon gas is used to prevent oxidation of the wine) are becoming very popular. The wine retains its characteristics for more than 3 weeks after opening the bottle, tasting as if it had just been opened. It offers the easy appeal of taste testing for a single glass of wine. In addition, the system can be set up with a wine card allowing a customer to serve themselves (such as in a boutique hotel bar without the presence of an on-site bartender). These systems are also very popular with consumers who prefer to be adventurous in tasting wines without dealing with a wine steward or bartender as they experiment with different wines.

Home Cocktail Systems

Similar to wine dispense systems, but designed for use in the home as a cocktail system ("smart bar" name OPN), a technology is being developed by Pernod Ricard for a 2018 launch. The concept is of a clever drink dispenser with six different spirit cartridges (replacing conventional bottles) and smart technology, which allows one to create perfect cocktails. It would also monitor and order supplies to ensure that you do not run out and could send you promotional offers and product updates. Designed to be an attractive-looking system, it would sit on a table replacing the group of bottles and mixes that you would normally require to make cocktails at home. The technology aspect also addresses the demand from consumers for smart technology products connected to the internet.

Labels and Decorative Cans

Advancements in the technology of label production for bottles are an interesting and creative area of innovation. Examples include labels that react to a variety of elements (temperature, UV light, moisture, black light, glow in the dark), conductive, holograph embossed, peelable labels, mold labels that can act as light barriers, tactile labels, and scratch and sniff labels. Labels can contain RFID for security or they can be created to interact with smartphones. The level of innovation on what is available in terms of label options for creative marketing has never been greater. Some labels are works of art that can stand on their own. The website www.ohbeautifulbeer.com is dedicated to celebrating graphic designs from the beer world, and it is interesting and delightful to browse.

Not only is there great innovation in labels that can be applied to bottles, but can technology also continues to evolve. The Molson Coors' Canadian unit, in collaboration with the crown plant in Batesville Mississippi, launched sun-activated ink on special Coors Light cans in the summer of 2017. The photochromic ink becomes visible in bright summery colors when exposed to UV rays and was launched in six limited edition designs and exclusive to the Canadian market. In addition, the use of a thermographic ink turns its mountain iconography blue when the can is cold (indicating it is ready to drink). The new cans use both ink technologies, and at 14 square inches in size, the thermochromic inks covered the largest-ever area to date on a beer can.

In addition to innovation in inks to decorate cans in new ways, there are cans that offer texture and sound. Kirin, with its Hyoketsu offering (a popular spirit and fresh juice mix), used a can where when opened, an embossed diamond pattern protrudes, emitting a sound, during the process, like crushed ice in a glass (due to the lost positive pressure in the can).

There appears to be no limit to the development of innovative cans in the marketplace. Legendary record label Island Records launched a session craft IPA beer, with the goal of creating beers that would complement their music theme. It won The World Beverage Innovation Awards 2017 for "best can." CROWN Bevcan worked with Island Records beer to supply a beer can that combined not only a soft varnish and cold feel to the can, but it complemented the consumer experience of drinking the beer with an app that scans the can (Shazam) and instantly links the consumer to a mood playing list that Island Records created on the music streaming service Spotify. These cans bring together a visual, touch, and sound experience before the consumer even opens and consumes the product, which is then further complemented by the taste and smell component of the beverage experience.

Edible Six Pack Rings

Plastic six pack rings for cans, although convenient for the consumer, have a very negative environmental impact on marine and other wildlife. A joint effort by a Florida-based brewery (SaltWater Brewery) and a New York City-based creative agency (We Believers) has resulted in a marine edible six pack ring, which is 100% biodegradable and compostable (www.e6pr.com). The ring resembles the recyclable pulp-fiber drink carriers used in coffee shops and is manufactured from brewery leftovers (wheat and barley). This product is expected to be on the market in the next 6–12 months and has already generated much excitement on social media sites in terms of the environmental benefits.

Other Fermented Products (Moving into New Geographic Regions)

Kombucha Kombucha is a fermented lightly effervescent sweetened black or green tea beverage. It is usually very low in alcohol and is believed to have originated in Manchuria over 2000 years ago. It is traditionally a home-brewed beverage but has been reborn as a trendy fermented product, rich in nutrients, with probiotic advertised benefits due to the symbiotic mixture of bacteria and yeast (known as SCOBY) used in its production. Commercially bottled kombucha is not new, but the market has shown strong growth in the past few years, and kombucha has now become mainstream in the USA. Many kombucha producers started as home brewers, later commercializing their offerings by partnering with distributors such as Whole Foods Market. Kombucha is now one of the fastest growing products in the US functional beverage market but is struggling in some states with the alcohol content designation (and therefore the tax situation), which can rise above 0.5% alcohol, due to the presence of the live organisms in the drink because, in certain cases, it can continue to ferment. The beverage, with many options for flavor additions, is well aligned with consumers searching for a different product, one that is considered to be a healthier choice than soda, especially with the current focus on the diet's impact on the human gut microbiome.

Baijiu (Unique Solid-State Fermentation Process) With the current globalization of products, one of particular interest is the Chinese distilled product baijiu, which has been produced for over 5000 years, mainly using sorghum as the substrate and a solid-state fermentation process. This is very different from the traditional liquid system used for beer, wine, and spirits. Rather than the alcohol being produced in a liquid tank environment, it is produced in a semisolid matrix and then the product is distilled and aged. In China, there are over 10,000 manufacturers who produce baijiu, using recipes inherited over time, and after distillation, the distillate is aged in earthenware jars or pits. Its production is a very unique and craft-driven process using techniques and bacterial and fungal cultures that have been handed down for thousands of years.

Although it is the most widely consumed spirit alcohol globally, outside of China, few people are familiar with it. Baijiu accounts for almost one-third of global spirit sales and is now the world's best-selling spirit. It has a very high alcohol content, typically between 40% and 60% alcohol by volume. "Maotai" the most famous baijiu is finding its way into markets outside of China and is being used as a special cocktail ingredient in trendy bars in North America in order to introduce the consumer to what is often termed as "firewater" in its undiluted form.

An excellent small book, written in English rather than Chinese, is titled "Baijiu: The Essential Guide to Chinese Spirits" by Derek Sandhaus (2014), which offers both historical and technological insights into this product which is unfamiliar to most of the western world. Although baijiu is certainly not a new product, it is a very new entry in terms of its movement to consumers outside of China.

Distilled but Nonalcoholic Beverages

Another interesting new entry into the beverage market is "Seedlip," which is a copper pot-distilled but nonalcoholic beverage. It is flavored with a mix of cold macerated and distilled botanicals, and it is marketed as a premium product to be used with various mixes in high-end bars and restaurants, in order to create a variety of nonalcoholic specialty drinks. Their marketing campaign uses the catch line: solving the dilemma of "what to drink when you're not drinking."

In a similar manner, Danish gin brand Herbie recently launched a nonalcoholic gin called Herbie Virgin gin. Their goal was to produce a product with the taste of gin, but without the alcohol, for the alcohol-free alternative market. Flavor ingredients of juniper, apples, lavender, and orange peel are distilled using water, in the same distillation equipment used for the alcoholic product, as they believe that a water distillation results in a more complex flavor and aroma than if you just allow herbs to draw in water without the distillation process.

Quality and Product Integrity

With the rise in craft brewing, and now craft distilling, one of the major problems brewers and distillers have faced in the past was that smaller operations cannot afford expensive laboratory equipment, or staff, to carry out analyses to ensure that processes are staying within established quality parameters.

Recently, due to the preponderance of smaller producers/home brewers, it has become a market large enough for equipment manufacturers to develop products for the laboratory including some that take advantage of a smartphone's data processing ability.

One such example of this type of new equipment is Anton Paar's EasyDens instrument, which is a density and extract meter targeted to home and small craft brewers. It links to the consumer's smartphone but still uses the same oscillating U-tube technology principle as with the larger industrial density meters used in most well-equipped brewery quality control laboratories. It allows the brewer to closely monitor the fermentation's progress and to know when the fermentation is complete without the use of a hydrometer.

Another innovative technology has been launched by Oculyze GmbH, a German company. They have addressed one of the most tedious jobs in a small microbiology laboratory, which is manual yeast viability cell counts using a microscope. It combines "methylene blue" (the time-tested favorite for staining cells), smartphone technology, and a web app. Using image recognition software, the product can determine the cell concentration, a budding cell count, and a culture's viability analysis in one measurement, in less than a minute. The smartphone microscope consists of a removable optical module (~400×), a smartphone, an app, and a connection to the server hosting the image recognition software. Users can access the Oculyze encrypted cloud platform from any location and review the yeast images and results. There is still the short step of diluting the yeast with the stain before it can be analyzed, but the counting process has been simplified in a very cost-effective manner for the brewer who may not be able to afford the more automated but fairly expensive cell viability instruments available on the marketplace.

Transparency and Labeling of Nutritional and Calorie Content of Alcoholic Products

Traditionally, the alcoholic beverage industry in terms of labeling has focused on alcohol content, but nutritional information and/or raw materials used in production were usually lacking. Alcoholic beverages have in the past been exempt from nutritional and allergen labeling in the majority of markets globally. Over the past few years, there have been changes in terms of the information presented on a label. Sometimes the changes are due to changing government rules, but more often they are due to acknowledging that the millennial consumer, in particular, demands more transparent product information. Whether that labeling is concerned with calorie and alcohol content, nutritional benefits, or sourcing of ingredients (i.e., no gluten, no GMO), a change is being seen across the alcoholic beverage categories. Diageo, in July 2017, was the first global alcohol beverage company to announce that they would be providing consumers with on-label alcohol and nutritional content. This transparency is being demanded by the consumer, but it also brings with it design issues on how to include all of the necessary information on one package. With the rapid advancement in interactive packaging technology, there will be many options outside the traditional label to help with this issue in the coming years.

Traceability

As technology continues to improve, the ability to track a product and then to interact with the end consumer continues to evolve. An example is CrownSecure™ from Crown Holdings Inc. A code scanning system assigns every single package its own unique and singular identity quick response (QR) code. The information is stored in a Cloud Datamatrix database, which can be accessed by retailers or consumers by scanning the code. This allows for the tracking of the product and for the validation of its authenticity (by watching for several scans in multiple locations). By imbedding unique digital intelligence into each individual unit, it makes the product simultaneously trackable and interactive.

Amcor Capsules has recently announced an anti-counterfeit smart capsule known as InTact, which uses a NFC (near-field communication) tag to transmit information on whether a bottle has been previously opened and resealed as well as further information about the beverage. The chip installed in the capsule that seals the bottle of wine or spirit interacts with an Android smartphone held near the neck of the bottle. It confirms authenticity while, at the same time, connecting with the consumer and giving information on production, history, serving information, etc. The product is hidden in an overcap, and therefore it does not disrupt the appearance of the original packaging. The current market target is premium wines and spirits, and the first product employing this technology will be a French estate wine.

Point-and-Shoot Technology for Product Liquid Fraud

Proactive anti-counterfeiting strategies are needed to safeguard not only brand reputations but also consumer safety. There have been many instances of harmful and potentially lethal products (mainly high levels of methanol) being illegally substituted into brand name bottles, which the customer had purchased trusting what they thought was a reputable name brand.

Researchers at the University of Manchester have developed the first handheld tool for alcoholic beverage analysis that uses a laser (Ellis et al. 2017). It can penetrate through materials such as colored glass and opaque plastic, yielding results within 1 min on the contents of the bottle. Using this technology, they were able to detect methanol (a poisonous compound) at levels below 0.025% in spiked test samples. The technology (SORS, spatially offset Raman spectroscopy) was initially developed to detect explosives hidden within glass and plastic bottles in airports.

Distilled Solutions, a Scottish company and a spin-off of M Squared Lasers, a company that also conducts work for the military, is developing a patented technique called modulated Raman spectroscopy, allowing for noninvasive identification of what is inside of the bottle, from outside of the bottle. Their eventual goal is a hand-sized instrument that can be used in the field. Once the liquid in the bottle is scanned, the scan can then be matched to a dataset built up by a fingerprinting device located in the production plant, where a scan was taken of the batch before it left the production plant. Any new spirit produced would be in the dataset and would be able to be matched in the field to confirm authenticity (Klaverstijn 2017).

A group of researchers from Heidelberg University in Germany recently published a paper on what they called a "synthetic tongue," a technology that uses different fluorescent dyes. When whisky is mixed with the dye, the brightness of the dye has subtle changes that allow one to see a highly specific profile for that particular whisky. A reference sample, alongside the test sample, allows one to definitively say whether it is an authentic sample or a fraud (Han et al. 2017).

The Future

One of the biggest challenges to the alcohol industry will be the rise of e-commerce. The buying experience will change dramatically when consumers are no longer required to be physically present in the retail environment to select a product from a display shelf. How will the new distribution channels affect how a consumer chooses their beverage? Globalization of products presents new opportunities for both the producers and consumers. The craft engine for both beer and spirits will continue to result in many changes in the marketplace as smaller producers can more easily experiment with product innovations and bring them rapidly to market.

Authenticity and quality however will always be the key to gaining consumer trust and repurchase. Today's consumer, with the prevalence of smartphone technology, is more educated and demands enhanced transparency from the industry in all aspects of beverage production and packaging.

References

Delgado-González MJ, Sánchez-Guillén M, García-Moreno MV et al (2017) Study of a laboratory-scaled new method for the accelerated continuous ageing of wine spirits by applying ultrasound energy. Ultrason Sonochem 36:226–235. https://doi.org/10.1016/j.ultsonch.2016.11.031

Ellis DI, Eccles R, Xu Y et al (2017) Through-container, extremely low concentration detection of multiple chemical markers of counterfeit alcohol using a handheld SORS device. Sci Rep 7:12082. https://doi.org/10.1038/s41598-017-12263-0

Forbes (2016) This July 4th, drink wine in a can (or a box, or a carton). 30 June 2016. https://www.forbes.com/sites/brianfreedman/2016/06/30/this-july-4th-drink-wine-in-a-can-or-a-box-or-a-carton/2/#39319b297da7

Han J, Ma C, Wang B et al (2017) A hypothesis-free sensor array discriminates whiskies for brand, age, and taste. Chem 2(6):817–824. https://doi.org/10.1016/j.chempr.2017.04.008

Klaverstijn T (2017) Could this little black box end fake whisky? 6 July 2017. https://scotch-whisky.com/magazine/in-depth/15119/could-this-little-black-box-end-fake-whisky/

Sandhaus D (2014) Baijiu: The essential guide to Chinese spirits. Penguin, UK

Chapter 6
Alcoholic Beverages: Technology and Next-Generation Marketing

Julie Kellershohn

Abstract Over the past 10 years, traditional marketing methods have expanded beyond print, billboard, radio, and television advertisements. The marketing world is continuously evolving with the generation of new technology-enhanced approaches, and with more options to choose from, marketers are expanding how they identify, target, and connect with various consumer groups. Topics covered include the impacts of the millennial generation, social media, authenticity, artificial intelligence, augmented and virtual reality, hyper-personalization, immersive experiences, loyalty programs, sensory marketing, visitor centers, and the voice of the brand.

Keywords Alcoholic beverages · Consumers · Hyper-personalization · Marketing · Millennials · Smartphones · Social media · Technology

Introduction: How the Millennial Generation of Consumers Are Changing the Role of Technology in Marketing

The millennial generation is referred to as the generation that came into early adulthood around the year 2000. By mid-2016, the millennial generation had overtaken the baby boomer generation as the largest living American generation, and the majority of this generation are now of legal drinking age. Millennial consumers tend to mistrust traditional approaches to advertising, and their views on national news media have grown more negative over the past 5 years (Pew Research 2016). Unlike prior generations, Millennials have a connection to gathering information from the digital world, with an inherent belief that the role of digital technology is to make their lives easier. As the most connected consumer generation in history, Millennials are highly addicted to the digital world, and the majority use two to three tech

J. Kellershohn (✉)
Ryerson University, Toronto, ON, Canada

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_6

Fig. 6.1 The millennial mindset

devices daily (Elite Daily 2015). The millennial connection with technology and their role as the consumer group with burgeoning buying power are changing the focus of the marketing industry, challenging marketers to expand their approaches to better address the habits and behaviors of the millennial consumer (Fig. 6.1).

As the generation that grew up with Starbucks, which uses customization to offer over 87,000 beverage options, Millennials view customization of products as an expected option, not as a unique and distinguishing attribute. Millennials have an expectation that brands will engage with them on social media, their preferred communication medium. While baby boomers are apt to purchase fine wines or spirits to drink for the "simple enjoyment of drinking," Millennials want the purchase to be part of a "social drinking experience" and will pay a premium price for a beverage with unique ingredients or a compelling brand story. Indeed, the use of digital communications gives marketers an opportunity to provide behind the scenes unique experiences that are only available due to the growth of technological options. The number one reason that Millennials post information about a brand or product online is the desire to share their opinion on the product's quality (Elite Daily 2015). To earn brand loyalty and brand advocacy with a millennial consumer, product quality is critical.

Having a Voice with the Brand

The millennial consumers seek out brands that will value their opinions, as demonstrated by brands that actively seek consumer feedback on new product development. While previous generations may have focused on convenience and price point when making a purchase decision, Millennials interweave the value of the experience into their decisions, especially with alcohol consumption decisions (Fona International 2016).

Wine consumption by Millennials shows a different trend from what has been seen in consumer patterns in past years. According to the Wine Market Council (www.winemarketcouncil.com), 36% of wine purchases in the USA are by millennial drinkers, and 17% of those millennial drinkers have paid over 20 USD a bottle compared to 10% of all drinkers. This behavior in wine purchase is also seen in other areas where Millennials value premium products and enjoy product exploration, all accelerated by the Millennial's affection for various technologies and smartphone apps.

Vivino is an excellent example of how smartphone apps can impact wine purchase decisions. Vivino is a web app that automatically tracks and organizes wines that you scan and rate. It helps you discover new wines and even lets you see how your choice ranks against your friends' choices. It is simple to use, as you only need to scan a label or wine list with your smartphone and the app immediately shows you the wine's ratings, reviews, and even the average pricing and the option to order (Fig. 6.2).

Crowdsourcing on Vivino offers you ratings on wines that have not been rated by the traditional wine rating experts and allows you to add your own opinion. There are over 23 million Vivino users and a listing of over 10 million wines and 65 million ratings (Forbes 2017). This is a very different model for how a consumer will intellectually decide on their wine purchase and will require a very different marketing approach to make a particular product stand out, when there are so many options.

Fig. 6.2 Using a smartphone app to obtain instant access to wine information and ratings

Stunt Marketing

Stunt marketing is the use of a promotional gimmick or publicity event designed to attract the public's attention, arranged primarily for media coverage and to drive consumer awareness of a product.

The Tequila Fountain Jose Cuervo recently put their Jose Cuervo silver tequila into three different water fountains in Los Angeles in honor of the National Tequila Day (July 24). After ensuring that consumers walking by, who wished to drink from the fountain, were over the age of 21, between noon and 6 p.m., they were free to drink from the fountain. This highlighted the fun nature of the product to differentiate it from the other tequila brands that more typically focus on refinement and conformity. It easily generated press interest and made for a perfect selfie-friendly social media shareable event.

The Opinions Welcome Campaign Laphroaig, an Islay single malt Scotch, launched a clever and hilarious campaign in 2014, which it now conducts yearly. It is their #OpinionsWelcome campaign, which plays on the brand's "love it or hate it" reputation. Consumers are invited to submit their tasting notes with the ability to win numerous prizes and trips to Islay for those deemed to have the best opinions. The brand says, “Whether you think our inimitable liquid is like having your ‘tongue pinched by a briney crab’ or tastes ‘smokier than Darth Vader’s funeral pyre’ get sipping.” The humor of the campaign plays well into the millennial experience of looking for unusual product tastes and works well with social media marketing (remembering that offering opinions and experiences is what Millennials value and enjoy).

Artificial Intelligence: Chatbots

Advances in technology enable brands and marketers to offer personalized experiences through artificial intelligent processes. Chatbots, which are apps driven by artificial intelligent processes, can conduct “natural” conversations with consumers and are becoming a powerful tool for brand marketers looking to engage customers more efficiently and effectively. Some analysts estimate that the introduction of chatbots may save companies up to 8 billion USD per year by 2022 (Juniper Research 2017).

Technology and Drinking/Eating Experience

In 2017, Bud Light debuted the Bud Light Touchdown Glass, offering their technology-enabled glasses for free, in specially marked cases and in some venues and for sale on their website. The glassware connects to a mobile app and lights up every time a fan’s favorite football team makes a scoring play; it’s about enhancing the game and beverage experience for the consumer.

To bring together a total drinking/eating and technology sensory experience to the consumer, there are affairs, such as the Stella Artois Sensorium Event, where the goal is to raise the premium perception of the brand. High-end dining provides an immersive experience for all five senses while highlighting the beverage and playing to all of the aspects that the millennial consumer values. It is touted as a once-in-a-lifetime special meal experience, with only limited tickets available. It is something that the consumer will want to share on their social media network. The first such Stella Artois immersive experience was held in Toronto in 2015 and included a multi-course gourmet meal prepared by a Michelin chef, with matched beers (sense of taste), a 360-degree film projected around the diners in a pop-up projection dome (sense of sight), hands on touching and preparing of some of the course ingredients (sense of touch), course-themed music (sense of sound), and waiters with misting bottles to provide specific aromas for the various stages of the evening (sense of smell). Gala events such as this using inventive sensory marketing and creative pop-up locations are becoming more commonplace as sensory marketing is used to link a product to a consumer.

In 2016, Malibu tested a number of connected drink cups that use the IoT (Internet of Things) technology to develop an on-demand drink delivery system in bars. It allows the customer to simply connect with the bar by twisting the bottom of the cup to signal staff. Different flashes from the cup let the customer know the order status. Wi-Fi and RFID technology, alongside a smartphone application, allow the bar staff to deliver the correct drink to the correct person (Internetofbusiness 2016a). Using similar technology, Martini trialed an IoT ice cube, which uses Bluetooth technology. It anticipates when your drink is finished and communicates with the bar in real time to ensure you have your refill, without ever having to queue in a line or try to catch a waiter's attention from a table (Internetofbusiness 2016b).

Technology and Loyalty Programs

Loyalty programs, designed to reward customers who frequently make purchases from a brand, are also experiencing a technology-driven evolution. The modern-day loyalty program is not simply the movement of a discount punchcard to an online platform, but rather it is an opportunity to reward loyal consumers, by creating unique offerings and learning more about the behavior of their customers in the process. AB InBev wanted to incentivize sales and build loyalty through brand engagement and rewards for their Busch beer brand. They launched Busch Bucks, a loyalty program in which consumers snapped a photo of their receipt for the purchase of Busch products and received points that they could exchange for items. They used the slogan "1. Buy Busch. 2. Collect Points. 3. Get Prizes." Additional support of the program was provided through their social media platforms, creating consumer conversations about how people were using their Busch Bucks. Consumers could trade points for consumer swag, such as a Busch golf shirt, and they also had a chance to win a 1 million dollar prize (Sports Business Daily 2017). By launching a digital loyalty program, AB InBev could receive consumer-level data on shopping patterns, such as frequency, payment method, store location, consumer age, contact information, e-mail, and social media accounts. This allows them to contact key target consumers for additional programs at a later date.

Heineken also launched a digital loyalty program but folded it into their beacon-equipped venues and GPS technology program. By installing Bluetooth beacons in 120 of its locations across New Zealand, consumers who had the mobile app on their iPhones had the opportunity to win big-ticket items such as helicopter transport to a special event and food and beverage vouchers each time they stopped by a branded outlet (Mobile Marketer 2016). From the marketer's perspective, creating a loyalty program that gave users points for going to venues and additional points for purchasing Heineken products, using beacon technologies, created an opportunity to bridge broader brand awareness into actual conversion, breaking new ground by creating a bridge from retail to bar.

Visitor Centers and Tasting Tours

Visitor centers, tasting tours, and road trips have long been an effective vehicle to tell a brand's product story to a large number of interested consumers. Visits that tour a number of producers and sample their products on such a route are an increasingly popular pastime for consumers.

This type of activity can help consumers form a connection to a brand, by offering them the experience of seeing how the product is made and tasting and learning about its unusual or interesting aspects. The Guinness Storehouse visitor center in Dublin educates the visitor with the story of one of Ireland's most iconic brands, Guinness beer. In 2015, it was named Europe's leading tourist attraction beating out the Eiffel tower, and in 2016 it hosted a record of 1.6 million visitors, exposing each of them to the taste and story of Guinness.

In the USA, the famous Kentucky Bourbon Trail tour, founded in 1999, in 2016 hosted over one million visitors. The Kentucky Bourbon Trail Craft Tour, which was added in 2013 to address the interest in the rise of craft bourbon distilleries, now includes 13 craft distilleries and hosted 177,228 trail visits in 2016, educating the consumer about bourbon in general and about varied brands and their unique processes (Kentucky Bourbon Trail 2017).

Scotland, with its five distinct whisky regions and whisky trails, each producing unique products to that region, and with more than half of the 123 Scotch whisky distilleries open to the public, attracted over 1.7 million visitors in 2016. Again, the growth and interest in craft distilling is very evident. Since 2013, 14 new whisky distilleries have opened in Scotland, and in 2017, it is projected that there will be an investment in 20 new distilleries, which will open within the next 2–3 years (Scotch Whisky 2017). With this abundance of choices for the visitors, how can a distillery make their site and product stand out? How can they appeal to the current boom in visitors from China and Russia (The Drinks Business 2017)? Some are using dining experiences with whisky and food pairings; others offer theme park-type rides in the facility. One of the most aspirational tours advertised is the Glenfiddich VIP tour – this is an exclusive VIP "behind-the-scenes" tour available only if you are a client of the private jet company, Air Partner. The concierge experience includes staying at the private Grant family house on the estate – a true "money can't otherwise buy" experience (and of course you can land your helicopter next door at the Balvenie distillery).

Technology has expanded the options for experiential marketing, now allowing consumers to tour locations before they are even built. When the Deschutes Brewery launched a campaign to build a new 85 million USD brewery, they first opened a taproom, inviting consumers to taste their products and offering a virtual reality experience in the taproom, where patrons could take a virtual tour of the brewery (The Bulletin 2017).

Virtual reality experiential marketing does not need to be limited to touring plants and production facilities; it is an opportunity to offer unique and memorable experiences from other locations as well. One Aldwych luxury hotel in London serves a 12-year-old Dalmore whisky cocktail (whisky mixed with cherry puree and grapefruit juice), and the drink is accompanied with the usage of VR goggles to transport the consumer to the Scottish Highlands location where the distillery is located to enhance their enjoyment of the cocktail (Vogue 2017).

Innis & Gunn has been experimenting with offering consumers virtual reality goggles (a Samsung VR headset and headphone combination), with the premise that they will taste the beer differently when they are immersed in their VR locations. They teamed up with Dr. Jolij, a cognitive neuroscientist, to create virtual reality content meant to take you from the sights and sounds of where you are located to various Scottish vistas. The goal is to trick the mind into thinking it is in that Scottish location and to taste the beer using those sights and sounds as the guide to tasting. The experience should trigger the brain to send new signals to the taste buds as you are no longer bound by being able to see the product you are drinking. Once you are desensitized to the clues that you usually use based on your taste perception memories, the theory is that your taste perception is therefore more open, allowing for more clinical and analytical thinking about the liquid, potentially leading to the discovery of new flavor aspects in the beer.

Customers Will Seek Out Authenticity

The Pearse Lyons Distillery in Dublin has taken a different approach to marketing its whiskey and attracting visitors to its boutique distillery, located in the historical district called “The Liberties.” This area of Dublin at one time was home to over 40 distilleries. This distillery, which opened for tours in 2017, is unusual in that it was built within a heritage site – the former St. James Church. The care and love that has gone into renovating and designing the site, to turn it into an operating distillery and visitor center, was a journey of love for the owners.

The distillery, impressive both inside and outside, and at a refurbishment cost of over 20 million USD, honors the heritage of the site with its custom-designed stained glass windows and its lit glass spire dome. The historic St. James Church site offers a unique learning experience to visitors, with intriguing tales of its history dating back to the twelfth century. The visit to the site is not only about distilling but about telling a story. When one visits, there is not only the visit to the distillery portion, but one learns about and visits the adjacent graveyard (where the owner’s own grandfather is buried) and learns the history of the church. The tours are kept small and never rushed. Each tour is about telling the story of the whiskey, the stories of The Liberties, and the history of the graveyard and making it very much a personal and memorable experience, which ends with a tasting of three samples of the distillery’s outstanding products. In a time when most visitor centers are using technology to enhance visits, this is a visitor center which heads in the opposite direction, relying on personal stories, small tour groups, and radiating authenticity. The visit is an unexpected wonderful surprise for visitors touching the heart, the brain, and the palate.

Customers Seeking Out the Unusual

The Speakeasy There is a revival of the speakeasy (the name given to hidden bars during the prohibition era). Hidden speakeasies are popping up in many cities, each with a unique theme and hard-to-find location, which makes the visit to it a very different experience. These locations, with their unique decors, appeal to consumers who enjoy the hidden aspect, the fun of discovering where the speakeasy is located and how to get into it, as well as the prohibition history aspect. Popular also for pop-up events, there are often distillers offering tastings of their products and using these events to market their product by word of mouth.

Bombay Sapphire Gin and the Grand Journey As consumers shop more and more online, they also still desire personal and immersive experiences, and pop-ups can provide that. Scale and creativity are seen in these pop-ups as witnessed by the Bombay Sapphire gin "Grand Journey" event. Bombay Sapphire gin recently used a fully functioning steam train to give customers such an experience – however, the train never left the station, but rather it transported the participants to different countries using lights, sounds, and tastes. A Michelin chef prepared a variety of dishes to match botanical-infused cocktails. During the journey, the ten botanicals in Bombay Sapphire gin were explored with a special cocktail and story behind each one. This allowed for a depth of knowledge about the gin to be transmitted in a unique and memorable format, and it was also presented in a way that was very sharable on social media. Pop-ups can provide an intimate brand-focused experience, where scale and creativity are unlimited.

At-Home Socializing: How Can I Create My Own Beverage or Experience?

Home Consumption Diageo, in their future trend study, identified that technology will continue to define at-home socializing (Diageo 2017). This trend can be seen with products and experiences available at the touch of a button (or order placed via e-commerce) and the proliferation of automated homebrew systems similar to Keurig-type coffee maker technology and home distillation systems. Customers are looking at options to make the home socializing experience about "at-home treating" with products such as adult alcoholic ice creams (wine ice creams and hard spirit ice creams).

Sensory at Home To enjoy at home, Courvoisier XO gift packs (Célébration Sensorielle) not only contain the bottles of cognac, but also heighten the flavor experience; there is the inclusion of fragrance cones (candles) meant to be lit while drinking the product. The intent is to make the experience Paris-inspired of the Belle Époque era, to take you back to a time that seemed simpler and full of elegance. One scent is candied oranges, patchouli, and tobacco (evoking Paris after dark), and one scent is vanilla, coffee, and fresh bread (evoking Paris by day). The inclusion of these scents, with their descriptions, adds a new level of sensory enjoyment to the drink occasion.

Augmented Reality and Virtual Reality Technology

There is expected to be a massive overall impact as augmented and virtual reality become an economic reality. Similar to the transformations in the economy from the impact of the personal computer, the Internet, and the smartphone, experts feel that AR and VR technology will have a similar effect on industries and economies (Fortune 2017). This influence will also be seen in their enhanced use in the marketing of alcoholic beverages. According to a study by Diageo (2017), the number of active VR users is forecasted to reach 171 million by 2018.

Augmented reality (AR) technology should not be confused with virtual reality technology (VR), as VR requires immersive headgear. Augmented overlays virtual 3D graphics onto our real world (augmenting), while virtual immerses us in a new synthetic world that has 360-degree views, with little or no input from the world we are actually in. Augmented technology is simpler with a lower barrier to entry; usually only a free-to-download smartphone app is needed.

Augmented technology has been used for a number of years and is not a new concept, but it is now expanding in both its usage and in its technological aspects. An example is the global mobile game phenomenon, Pokémon Go, whose app to date has been downloaded more than 750 million times worldwide. Players use their smartphones to hunt digital creatures that are overlaid onto the real world with the help of their phones' sensors and cameras. The popularity of this game showed that augmented reality experiences can engage massive audiences and brought augmented reality into the mainstream. It is expected that AR and VR will generate 150 billion USD in revenue by 2020. The increasing power of smartphones, where filters and animations can be added to people's photos (e.g., Snapchat) and videos and where it is possible to overlay geo-specific content on live streams, now allows for the creation of unique experiences where the digital and real worlds merge.

Music and AR Experiences Shazam is one of the world's most popular free music apps, and the song identifier app has been downloaded over 1 billion times, in over 190 countries, and users *Shazam* over 20 million times each day. Shazam has been working with companies to create augmented reality apps for their promotions. In the spring of 2017, Shazam announced the launch of its new augmented reality (AR) platform for its brand partners, artists, and global users. One of those launch partners is Beam Suntory, the world's third largest premium spirits company. The new platform can bring different marketing materials to life. The customer uses the app to scan the unique Shazam code, which will then deliver AR experiences including 3D animations, product visualizations, mini-games, and 360-degree videos. It is all about the immersive experience.

Marketing Through Smartphones

Technology has become an integral part of how the millennial consumer shops; providing easily accessible, mobile-friendly information to a tech-savvy consumer base can pay off. While conventional thinking has noted that price and recommendations from friends/family have been the most important factors influencing purchase decisions in the past, there is a growing role for online information which has now become one of the major influencing factors. The growth of mobile shopping apps is also changing how consumers shop. Because of their affection for technology, Millennials are generally more highly engaged in prestore buzz than older generations. Consumers are looking for easy-to-read content that can be read on the go and that enables them to engage with the product and make quick and informed purchase decisions.

Social Media

Millennial consumers are more trusting of digital media than the general population, with an estimated 66% of Millennials trusting search engines as their source of news and information (Edelman 2016). Largely evolved past the traditional marketing approach of celebrity-endorsed products, social media campaigns are now more focused on creating an online buzz through interactive opportunities and the pursuit of campaigns so successful that they go viral, an oft sought but rarely achieved status. In the digital marketing space, consumers share their personal experiences with their audiences on Facebook, WeChat, Twitter, and Instagram, as well as other social media platforms. With an average of 3 h or more spent per day on social media, the millennial consumer is tuned into social media messaging. The brands do not control these individual consumer-led brand communications, and yet they have the potential to be both beneficial and detrimental to brands.

Marketers are turning to a variety of social media platforms to connect with consumers in the digital environment that is part of their everyday life. For example, the marketing team at Three Olives Vodka partnered with dating app Tinder, offering matched couples a $2 rebate on any Three Olives cocktail for their first date (Marketing Daily 2017).

Brick and Mortar stores are finding new ways to bring social media into their stores, knowing that the majority of young consumers say they are more likely to make a purchase when influenced by a social media reference.

While not easy to achieve, when a social media campaign goes "viral," the consumer exposure that a strong idea or compelling story can achieve is significantly beyond what a typical marketing budget could afford for media exposure. For example, when the creative agency "We Believers" created a campaign around a compostable, biodegradable, and edible beer can holder ring, meant to replace the traditional plastic ring on six packs of beer and soda cans, the campaign surpassed the reach of a typical marketing campaign. The video of their product innovation had over 250 million views on Facebook and over 8 billion global impressions (UVAToday 2017).

Perceived Healthier Alcoholic Products

Alcoholic juice is a product concept that combines the current desire for healthy beverages with the desire for alcoholic content and works off the healthy juice bar principal offered in many gyms. In the UK, the retailer “Supernatural” (www.sprntrl.co.uk), advertised as the UK’s first alcoholic juice bar, sells nonalcoholic drinks during the day but alcoholic options in the evenings. It is located close to a fitness studio to appeal to the gym-oriented consumer. Examples of their product include a rum drink called a Piña Kale-ada (rum, fresh kale, coconut milk, kale syrup, date syrup, citrus, and smoked sea salt) and a vodka drink called the Beetrooter (vodka, beetroot, apple, ginger, agave, lime, blueberries as garnish). Whether this concept will expand is still to be seen, but it is aligned with the move toward healthy and unique beverages, a millennial oft-articulated wish. Along with this concept of healthier alcoholic products comes the wide range of products that are promoted and marketed as “healthier for you” (organic, locally grown, non-gluten, non-GMO, non- or low-sugar content, lower alcohol, etc.).

Shopping Behaviors and Technology

Their relationship with technology has shifted how Millennials buy products. Using technology to stay connected while shopping, they can easily reach out to family and friends for opinions, read online product reviews, comparison shop for price, and instantly share their own experiences online to influence others.

For a generation of consumers that view the role of technology as a means to make their lives easier, developments in e-commerce are changing how marketers connect with consumers. They can create an at-home shopping experience superior or easier than an in-store experience. MillerCoors and IPG Media Lab partnered with an on-demand alcohol delivery service, Drizly, to develop a branded Amazon Dash button for 500 customers, as well as an Amazon Alexa skill with the trigger phrase “Start Miller Time” (MediaPost 2017). While still in the pilot phase of testing, the project is designed to bring easy 1-h beer delivery to people’s homes.

Dom Pérignon is testing a 1-h delivery service in select cities in the USA. It offers its vintage products directly from its site, where customers choose a desired bottle and enter a delivery address and payment method, and the order arrives chilled and ready to drink within the hour (PR Newswire 2017).

In the digital age, information is being discovered and disseminated at extraordinary rates, and you do not need to be a big player in the industry to attract customers from across the country or even across the world. The introduction of flash sales has also changed how some consumers purchase online. A flash sale is a discount or promotion offered by an e-commerce store for a short period of time. The product quantity is limited, which often means the discounts are higher and there is a time limit (often 24 h or less). Limited availability drives impulse buying amongst consumers. Flash sales originated in brick and mortar stores as ways to unload overstocked merchandise. This approach has moved online, with companies that only offer flash sales with rapidly changing consumer offerings. Invino (www.invino.com) offers daily sales events, with wines sold at up to 70% off retail. What flash sales lack in consistent and guaranteed selection, they make up to consumers by offering heavily discounted products. They often work directly with wineries to offer consumers another option for how to purchase products.

Better Consumer Insights Through Innovative Uses of Technology

Understanding the needs and wants of the consumer is critical information to any marketer. By understanding what the consumer wants, products and campaigns can be created that connect to the consumer's interests. Keeping focused on the elements most essential to the target consumer saves money, time, and energy by not draining resources supporting elements that the consumer does not value. However, consumer research is not infallible, as human behavior can be challenging to predict. Advances in how technology is used to conduct consumer research have helped to evolve what we know about human behavior and how marketers can better connect with their target market. From traditional ethnographic studies (Diageo 2017) to help us understand purchasing behaviors to brain scans that explain why the identical wine tastes better to participants when labeled with a higher price tag (Schmidt et al. 2017), clearly there are many techniques that a marketer can invoke.

Personalization and customization are two trends that the app Vinfusion (being developed by the product design and development firm Cambridge Consultants) addresses. It allows customers to personalize their wine preference by using simple terms such as full-bodied or light, dry, or sweet. Then four base wines are blended using their flavor algorithm to blend and dispense a wine personalized for the consumer on demand (Cambridge Consultants 2017). The next stage in the development of this concept is their addition of machine vision technology to obtain consumer insights. For example, their results suggest that males appear to prefer fuller-bodied fiery wines, while females prefer softer lighter wines – information obtained without interviews or questionnaires. When an individual tastes the unique wine that has been blended based on their entry into the connected mobile app, the image recognition technology assesses whether the individual has enjoyed the wine by analyzing their facial reaction immediately after tasting. Gender and age are also determined by the visual recognition technology allowing for additional analysis of the results. Will facial recognition technology result in a better understanding of consumer preferences, and will it be a tool that marketers routinely use in the future? Interesting question. Recent publications (He et al. 2017; Yu and Ko 2017) describe experimentation that uses facial expressions and FaceReader technology by Noldus (www.noldus.com), suggesting that it is a technology that needs to be explored further by researchers to determine it applicability.

Hyper-personalization It is foreseeable that in the future customers may well create their own drinks in micro-runs, either through websites or tasting sessions in tap rooms and venues, such as those described above by Vinfusion. Hyper-personalization can be seen in the marketing approach by the wine company Vinome (www.vinome.com). They ask the question "What if wines could be scientifically selected for you based on your DNA?" and then offer you the ability to purchase a DNA kit with your wine to have your DNA analyzed for specific genetic markers. Your DNA results will tell you about the presence or absence of certain alleles, which give a peek into characteristics such as how sensitive are you to bitter flavors and do you have a genetic sweet tooth. Although at this stage the technology for associating genetics and flavor preferences is still at a very early stage (they only test for ten genetic markers), it does give us a glimpse into the future of what may be possible some day in terms of how our DNA could provide interesting insights into our beverage preferences, though the question remains how willing will the consumer be for hyper-personalization with something as personal as DNA analysis, which, if not kept private, could be used for other purposes, such as denial of health insurance in some future scenario.

The Future

Consumers are demanding ever-increasing levels of personalization in their products, increased convenience, eco-sensitivity, and authenticity. They have no tolerance for any compromises in terms of quality. Instant gratification with the ability to make it an Instagram-able experience, a memory to keep, is what today's millennial consumer is looking for. Packaging is more important than ever and can be used to engage the tech-savvy Millennials and deliver personalized drinking experiences and demonstrate how innovative a company is. Both playful and premium packaging will continue to have great appeal. E-commerce will become a massive disruptive force in the industry, as it is not just a sales channel but it is also quickly becoming a crucial marketing channel, requiring its own strategies. However, we also know that the consumer still wants contact with the brand and that options such as pop-up events provide this, and these are expected to be even more innovative than what has been seen in the past. It is an exciting time for marketers; with updated technology and apps coming onto the market daily, gaining the customers' allegiance to one product or brand has never been more difficult.

References

Cambridge Consultants (2017) The future of consumer insights. 11 Sept 2017. https://www.cambridgeconsultants.com/media/press-releases/future-consumer-insights

Diageo (2017) The changing face of socializing. 18 Jan 2017. https://www.diageo.com/en/news-and-media/press-releases/the-changing-face-of-socialising/

Edelman (2016) The 2016 Edelman trust barometer—slide 45. http://www.edelman.com/insights/intellectual-property/2016-edelman-trust-barometer/global-results/

Elite Daily (2015) Elite Daily Millennial consumer study 2015. 19 Jan 2015. http://elitedaily.com/news/business/elite-daily-millennial-consumer-survey-2015/902145/

Fona International (2016) The 2016 trend insight report. Millennials: alcoholic beverages/spirits/beers. http://www.fona.com/sites/default/files/Millennials_alcoholic%20beverages_0116.pdf

Forbes (2017) The launch of Vivino market could herald a new era in wine buying. 30 Mar 2017. https://www.forbes.com/sites/brianfreedman/2017/03/30/the-launch-of-vivino-market-could-herald-a-new-era-in-wine-buying/#1de4059b5ed1

Fortune (2017) Augmented reality may reinvigorate these three industries. 21 Sept 2017. http://fortune.com/2017/09/21/augmented-reality-3-industries/

He W, Boesveldt S, Delplanque S et al (2017) Sensory-specific satiety: added insights from autonomic nervous system responses and facial expressions. Physiol Behav 170:12–18. https://doi.org/10.1016/j.physbeh.2016.12.012

Internetofbusiness (2016a) Malibu IoT cup makes bar queues a thing of the past. 20 Oct 2016. https://internetofbusiness.com/malibu-iot-cup-queuing-bar/

Internetofbusiness (2016b) Martini launches IoT ice cube in bid to shake up the drinks industry. 5 Oct 2016. https://internetofbusiness.com/martini-iot-ice-cube-drink/

Juniper Research (2017) Chatbot conversations to deliver $8 billion in cost savings by 2022. 24 Jul 2017. https://www.juniperresearch.com/analystxpress/july-2017/chatbot-conversations-to-deliver-8bn-cost-saving

Kentucky Bourbon Trail (2017) Kentucky Bourbon Trail barrels past one million visits in 2016. 25 Jan 2017. http://kybourbontrail.com/kentucky-bourbon-trail-barrels-past-1-million-visits-2016/

Marketing Daily (2017) Michelob Ultra offers workout bot; Three Olives rewards Tinder users via iBotta. 3 Aug 2017. https://www.mediapost.com/publications/article/305321/michelob-ultra-offers-workout-bot-three-olives-re.html

MediaPost (2017) MillerCoors, IPG create one-button ordering for beer. 23 Mar 2017. https://www.mediapost.com/publications/article/297686/millercoors-ipg-create-one-button-ordering-for-be.html

Mobile Marketer (2016) Heineken uncaps experience-driven rewards for app users via beacons. 7 Jul 2016. http://www.mobilemarketer.com/ex/mobilemarketer/cms/news/strategy/23183.html

Pew Research (2016) Millennials' views of news media, religious organizations grow more negative. 4 Jan 2016. http://www.pewresearch.org/fact-tank/2016/01/04/millennials-views-of-news-media-religious-organizations-grow-more-negative/x`

PR Newswire (2017) Dom Pérignon partners with Thirstie to launch its first on-demand delivery pilot at domperignon.com. 27 Jul 2017. http://www.prnewswire.com/news-releases/dom-perignon-partners-with-thirstie-to-launch-its-first-on-demand-delivery-pilot-at-domperignon-com-300495062.html

Schmidt L, Skvortsova V, Kullen C et al (2017) How context alters value: the brain's valuation and affective regulation system link price cues to experienced taste pleasantness. Sci Rep 7(1):8098. https://doi.org/10.1038/s41598-017-08080-0

Scotch Whisky (2017) Scotch whisky distilleries to open in 2017. 5 Jan 2017. https://scotchwhisky.com/magazine/features/12315/scotch-whisky-distilleries-to-open-in-2017/

Sports Business Daily (2017) Anheuser-Busch launches BuschBucks.com as part of new marketing, loyalty campaign. 18 Apr 2017. http://www.sportsbusinessdaily.com/Daily/Issues/2017/04/18/Marketing-and-Sponsorship/Busch.aspx

The Bulletin (2017) Hundreds attended Deschutes Roanoke pub opening. 31 Aug 2017. http://www.bendbulletin.com/business/breweries/5557368-151/hundreds-attended-deschutes-roanoke-pub-opening
The Drinks Business (2017) Brexit gives boost to Scotch whisky tourism. 8 Sept 2017. https://www.thedrinksbusiness.com/2017/09/brexit-gives-boost-to-scotch-whisky-tourism/
UVAToday (2017) Saving sea turtles, one six-pack at a time. 28 Aug 2017. https://www.news.virginia.edu/content/saving-sea-turtles-one-six-pack-time
Vogue (2017) There's now a virtual reality cocktail (and yes, you do get a real drink). 23 Aug 2017. https://www.vogue.com/article/virtual-reality-vr-cocktail
Yu CY, Ko CH (2017) Applying FaceReader to recognize consumer emotions in graphic styles. Procedia CIRP 60:104–109. https://doi.org/10.1016/j.procir.2017.01.014

Chapter 7
Advances in Probiotics, Prebiotics and Nutraceuticals

Swati S. Mishra, Prafulla K. Behera, Biswabandita Kar, and Ramesh C. Ray

Abstract The chapter deals with the advances in technologies for industries related to the production of probiotics, prebiotics and nutraceuticals. The novel innovations in important produces such as milk-based products, fruit- and vegetable-based products, root- and tuber-based products, dried probiotics and beverages are elucidated. Further, the latest technological interventions in nanotechnology, microencapsulation and immobilization techniques with respect to the functional food industries are presented. The chapter also describes the food ethics, safety, regulations and the functional food market.

Keywords Fermented foods · Functional food · Prebiotics · Probiotics · Synbiotics · Nutraceuticals · Immobilization · Nanotechnology · Food ethics · Food market

Introduction

Let food be thy medicine, thy medicine shall be thy food. Hippocrates

The pace of life has become very fast and so also the change in food habits. Health and longevity have become the new *mantras* (consciousness) in people. The health benefits of certain foods containing bioactive compounds (matrices having positive effects on human health) have been realized for preventing many diseases (El

S. S. Mishra (✉) · P. K. Behera
Department of Biodiversity and Conservation of Natural Resources, Central University of Orissa, Koraput, India

B. Kar
School of Applied Sciences, KIIT University, Bhubaneswar, India

R. C. Ray
ICAR-Regional Centre of Central Tuber Crops Research Institute, Bhubaneswar, India

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_7

Sohaimy 2012; Palozza et al. 2010). These compounds or foods used in promoting health are classified either as functional foods or nutraceuticals. These foods have been claimed to render health benefits and are marketed as functional foods. The trillion of microbes colonizes in the human body (Martıín et al. 2013), some of which are beneficial, whereas others are harmful. An imbalance between the beneficial and harmful bacteria leads to diseases such as obesity and urinary tract infections (Vujic et al. 2013). Hence balancing the so-called good bacteria in our gut in day-to-day life activity is of prime importance. The functional foods impart health promotions by food supplements, i.e. probiotics, prebiotics and synbiotics that help in altering and reinstating the pre-existing intestinal flora (Pandey et al. 2015).

The health benefits imparted by these functional food aim to create foodstuffs which after ingestion multiply "healthy" bacteria in the intestine. Till date, Japan is the only country that has formulated a specific regulatory approval process for functional foods. Japanese coined the term Foods for Specified Health Use or FOSHU, to which a functional ingredient has been added for a specific healthful effect (Berry 2002; Kaur and Das 2011). Growing interest among consumers has led to the development of modern technology for innovation or interventions in production of prebiotics, probiotics and nutraceuticals in order to enhance the life span and quality of human beings. Nowadays, several innovative technologies such as immobilization, nanotechnology and encapsulation are being used for the production of probiotics, prebiotics and nutraceuticals.

The functional foods and nutraceuticals provide an opportunity for betterment of health, cut healthcare costs and support economic development (Wildman 2001; Takayuki et al. 2008). There are many challenges like country-wise regulations, difficulty in health claim substantiation and low innovation by food companies that makes the future of these functional food markets difficult to predict (El Sohaimy 2012). Despite many useful benefits of functional foods and nutraceuticals, many legislative problems occur in different countries as we are consuming live organisms. This chapter provides an overview of different technologies for the production of functional foods, i.e. prebiotics, probiotics, synbiotics and nutraceuticals.

History, Definitions and Sources

Functional Foods

Although different definitions for functional foods are used worldwide, there is no official or commonly accepted definition that exists, so far. However, broadly any food accompanying a particular health claim can be defined as functional foods. The concept of functional food was invented in Japan during the 1980s, when health authorities realized the need for enhancement in the life quality and expectancy so as to reduce the healthcare costs (De Sousa et al. 2011; El Sohaimy 2012). It emphasizes that food is not only vital for living but also source of physical and mental well-being contributing towards prevention and reduction of risk factors for several diseases. Functional foods are essential for enhancing physiological functions and to provide the body with required nutrients (Cencic and Chingwaru 2010; Lobo et al. 2010).

Functional foods are categorized as (i) foods with naturally occurring bioactive substances (e.g. dietary fibre), (ii) foods supplemented with bioactive substances (e.g. probiotics) and (iii) derived food ingredients introduced to conventional foods (e.g. prebiotics), through a combination of probiotics and prebiotics (e.g. synbiotics). Probiotics, prebiotics, synbiotics, vitamins and minerals: coming under functional food and currently used for human consumption in the form of fermented milks and yoghurts, sports drinks, baby foods, and chewing gum (Figueroa-Gonzalez et al. 2011; Al-Sheraji et al. 2013).

Probiotics

Although probiotics are associated with gut health, the history of probiotics dates long back. Albert Döderlein was first to suggest the beneficial association between microorganisms and the human host, and in 1892, he postulated the vaginal bacteria produce lactic acid that inhibited the growth of pathogenic bacteria (Döderlein 1892). In 1908, Ilya Metchnikoff was the first to speculate the beneficial properties of lactic acid bacteria (Metchnikoff 1908). The work of Minoru Shirota, who was first to actually cultivate *Lactobacillus casei* Shirota, a beneficial intestinal bacterium and distributed in dairy drink that was introduced to the market in 1935, was another milestone in the development of probiotics (Yakult Central Institute for Microbiological Research 1999).

The increase in knowledge with respect to their function modified the definitions of probiotics. The term “probiotics” is derived from the Greek word meaning “for life” and was originally proposed to describe growth-promoting substances produced by one protozoan for the benefits of another (Lilly and Stillwell 1965). Further, in 1974, the definition of probiotics was given by Parker as “organisms and substances which contribute to intestinal microbial balance” (Schrezenmeir and de Vrese 2001). It was later redefined by Fuller in 1989 as “food supplemented with live microbes that benefits host animals by improving its intestinal microbial balance” which was the first generally accepted definition (Fuller 1989; Khan and Naz 2013; Panda et al. 2017). Nowadays, a widely accepted broad definition that was proposed by a Joint Expert Consultation of the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) classified probiotics as “live microorganisms that, when consumed in an adequate amount, confer a health benefits on the host” (FAO/WHO 2001).

Sources and Microorganisms Used as Probiotics

The common sources of probiotics are yoghurt, cultured buttermilk and cheese. The bacterial fermented foods are Japanese miso, beer, sour dough, bread, chocolate, kimchi, olives, pickles, tempeh and sauerkraut. But among these, the dominant foods in probiotics are still yoghurts and fermented milks, as they have a relatively low pH environment for the survival of probiotic bacteria (Anandharaj et al. 2014). The genera *Lactobacillus* and *Bifidobacterium* comprise majority of probiotic microorganisms. Lactic acid bacteria are commonly used as probiotics. Lactobacilli are non-spore-forming rod-shaped bacteria. They have complex nutritional requirements and are strictly fermentative, anaerobic and acidophilic. Majorly the human intestinal microflora constitutes the bifidobacteria (De Vrese and Schrezenmeir 2008). Some of the popularly used probiotic microorganisms are *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, bifidobacteria and certain strains of *Lactobacillus casei*, *Lactobacillus acidophilus*-group, and *Bacillus coagulans*; *Escherichia coli* strain Nissle 1917; certain enterococci, especially *Enterococcus faecium* SF68; and the yeast *Saccharomyces boulardii* (Pandey et al. 2015). Other lactic acid bacteria from genera such as *Streptococcus*, *Lactococcus*, *Enterococcus*, *Leuconostoc*, *Propionibacterium* and *Pediococcus* are also now included in probiotics (Krasaekoopt et al. 2003; Power et al. 2008).

Prebiotics

Gibson and Roberfroid defined probiotics in 1995 as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health.” FAO/WHO defines prebiotics as a non-viable food component that confers health benefit(s) on the host associated with modulation of the microbiota (FAO/WHO 2001). An ideal prebiotic should be (1) resistant to the actions of acids in the stomach, bile salts and other hydrolysing enzymes in the intestine, (2) should not be absorbed in the upper gastrointestinal tract, (3) should be easily fermentable by the beneficial intestinal microflora (Kuo 2013).

Sources of Prebiotics

Some sources of prebiotics are raw oats, soya beans, unrefined barley, breast milk, yacon, non-digestible carbohydrates, non-digestible oligosaccharides, etc. However, only bifidogenic, non-digestible oligosaccharides, particularly inulin, its hydrolysis product oligofructose and (trans) galacto-oligosaccharides (GOS), fulfil all the criteria for prebiotic classification (Pokusaeva et al. 2011).

Synbiotics

The additional benefit when prebiotics is combined with probiotics was speculated by Gibson after he introduced the concept of probiotics. This combination of prebiotics and probiotics he termed as synbiotics (De Vrese and Schrezenmeir 2008). Thus “synbiotic” beneficially affects the host in improving the survival and selectively stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria in the gastrointestinal tract. The products in which the prebiotic compound(s) selectively favour the probiotic organism(s) are true synbiotics (Cencic and Chingwaru 2010). It also helps to overcome the possible survival difficulties for probiotics. Fermented milk is considered synbiotics as it provides both live beneficial bacteria (probiotics) and products of fermentation that may affect the intestinal microflora in a positive way (prebiotics) (Famularo et al. 1999).

Sources of Synbiotics

For synbiotics formulation, the probiotic strains used include *Lactobacillus*, *Bifidobacterium* spp., *Saccharomyces boulardii*, *Bacillus coagulans*, etc., while the major prebiotics used comprise of oligosaccharides like fructo-oligosaccharide (FOS), galacto-oligosaccharides (GOS) and xylose-oligosaccharide (XOS), inulin, prebiotics from natural sources like chicory and yacon roots, etc. The main health benefits of synbiotics are (1) increase in the balance of gut microbiota, (2) improvement of liver function in cirrhotic patients, (3) improvement of immune-modulating ability, (4) prevention of bacterial translocation and reduced incidences of nosocomial infections in surgical patients, etc. (Zhang et al. 2010)

Nutraceuticals

The term “nutraceutical” was coined from the words “nutrition” and “pharmaceutical” in 1989 by Stephen DeFelice. He defined nutraceuticals as “a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease” (El Sohaimy 2012). They are usually in the form of capsules, pills and tinctures. Nutraceuticals cover a broad range of products including beverages, dietary supplements, isolated nutrients, genetically engineered designer foods, herbal products and processed foods. The basic categories of nutraceuticals are (1) dietary supplements, (2) functional foods and beverages and (3) nutraceutical ingredients (raw minerals or oils) (El Sohaimy 2012).

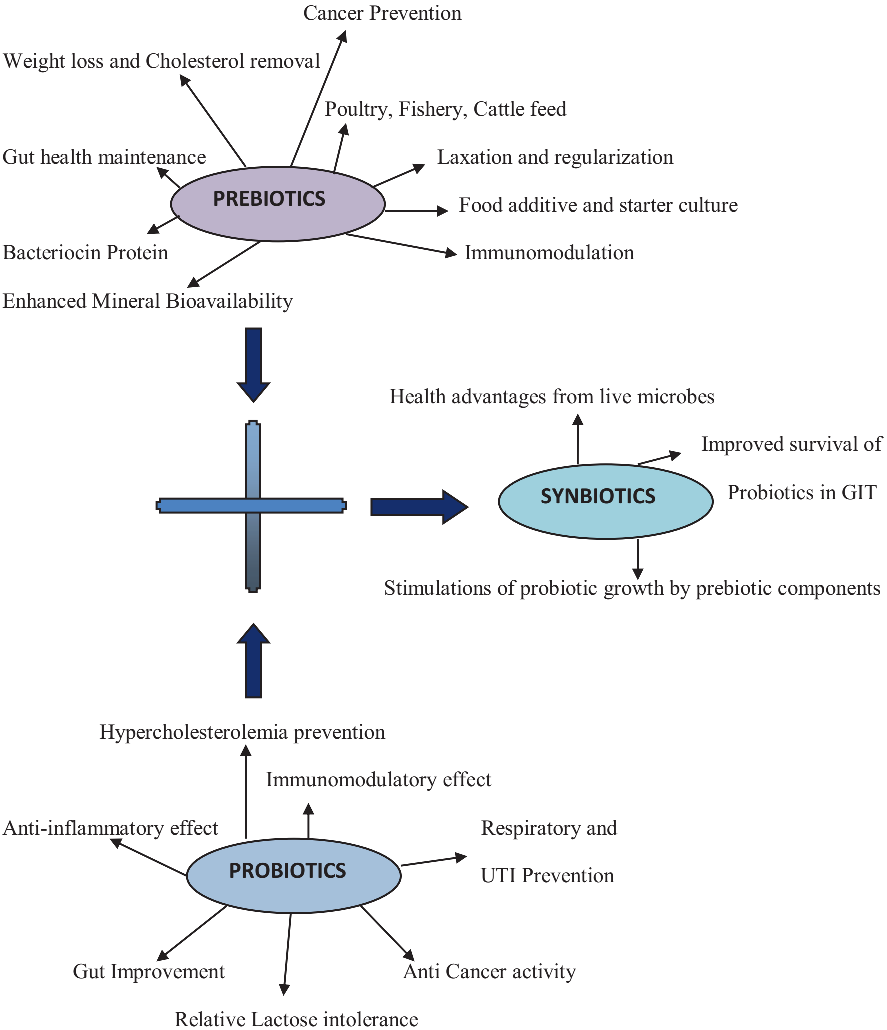


Fig. 7.1 Pictorial summary of prebiotics, probiotics and synbiotics and its uses

Probiotics in Food Processing/Fermented Foods

Probiotics in food not only adds to palatable delights but also gives higher health benefits (Fig. 7.1). However their high stability during production and storage throughout the whole shelf life to maintain the probiotic effect of the food is highly important. Various probiotic products are discussed below with respect to their base substances.

Milk-Based Products

Probiotic milk products, fresh cheese and ripened cheese are well-established products. Prebiotic lactulose in skimmed milk increases the probiotic counts (particularly *Bifidobacterium lactis*), the acidification rate and lactic acid concentration and also reduces the fermentation time (Oliveira et al. 2010). Milk whey culture is a useful prebiotic for the therapy of inflammatory bowel disease (Uchida et al. 2007). Probiotic ice cream is known since the 1960s. Probiotic starter cultures, yoghurt powder and fermented milk products are added for making these ice creams. This ice cream supplemented with *Lactobacillus casei* and 2.5% inulin showed good nutritional and sensory properties (Criscio et al. 2010). Probiotic variants of other fermented milk products like sour milk, sour whey, sour cream, buttermilk or kefir are not very popular. In Europe, unfermented milks with added probiotics (sweet acidophilus milk, bifidus milk) are much less popular than yoghurt.

Cereal-Based Products

Cereals, in particular oat and barley, are important for the production of functional foods. The multiple beneficial effects of cereals can be exploited in different ways leading to the design of novel cereal foods or cereal ingredients that can target specific populations. Cereals ("flakes"), added with sugar and lyophilized probiotic cultures, were used as a simple, direct delivery vehicle for dried probiotics (Sanders 2003). Dietary fibres give breads with better sensory perception, lower digestible starch and higher resistant starch contents (Angioloni and Collar 2011). Damen et al. (2012) investigated the in situ production of prebiotic AXOS during bread making. Mitsou et al. (2010) evaluated the in vivo prebiotic potential of barley β-glucan and concluded that it induced a strong bifidogenic effect.

Fruit-/Vegetable-Based Products

Fermented vegetable products (e.g. "sauerkraut", kimchi or "lacto-pickles") contain live lactobacilli, having probiotic health effects (Swain and Ray 2016). *Lactobacillus johnsonii* B-2178 is used to evaluate the probiotic effects of fermented cashew and apple juice (Vergara et al. 2010). Ro¨ßle et al. (2010) developed a potentially synbiotic fresh-cut apple wedges by applying probiotic bacteria (*Lactobacillus rhamnosus* GG) and prebiotics oligofructose and inulin.

Roots and Tuber-Based Products

Sinki and sunki It is a fermented radish-based product, native to India, Nepal and Bhutan. The predominant probiotic bacteria found are *Lactobacillus fermentum*, *Lb. brevis* and *Lb. plantarum* (Swain et al. 2014). The Japanese product "sunki" is made from a non-salted fermentation process of otaki-turnip (Battcok and Azam-Ali 2001). The bacterial community were stable, and *Lactobacillus delbrueckii*, *Lb. fermentum* and *Lb. plantarum* were dominant during the fermentation (Endo et al. 2008), when profiled by PCR-denaturing gradient gel electrophoresis.

Kanji The traditional product "kanji" made in northern India and Pakistan is prepared from fermented purple carrots. The grated carrots are subjected to brine salted fermentation for 7–10 days with addition of water. From carrot kanji two distinct genotypes of *Lb. paraplantarum* and one genotype of *Lb. pentosus* were identified (Kingston et al. 2010).

Dried Probiotic Products

Spray-dried probiotic bacteria can be applied directly in the manufacture of sweets, confectionary pastries and infant foods (Corcoran et al. 2004). Alternatively milk powder (skimmed milk, whey, buttermilk or yoghurt powder) can be used as a delivery system. Less study has taken place for the stability of probiotic bacteria in powdered milk products (Gardiner et al. 2000).

Beverages

One important functional food is non-alcoholic beverages fortified with vitamins A, C and E or other functional ingredients. There are many cholesterol-lowering drinks (with combination of omega-3 and soy), "eye health" drinks (with lutein) or "bone health" drinks (with calcium and inulin) (Keller 2006) as functional foods as beverages. In Estonia, for example, fortified juices are produced under the trade name of Largo containing inulin, L-carnitine, vitamins, calcium and magnesium as functional ingredients (Tammsaar 2007).

Technological Intervention in Functional Foods

Technological aspects in microbial systems and functional foods consist of many steps – composition and processing of raw materials, the viability of used starter cultures and technological and storage conditions of the final foods. Discussed below are the technological interventions in developing functional foods.

Nanotechnology

The novel technology having the potential to revolutionize the food systems is nanotechnology (Huang et al. 2010). Large surface area and small nanoparticles are useful for potential application of nanotechnology in the field of food sectors. It also involves nutraceuticals within nanocapsules, nanoencapsulated flavour enhancers (Siegrist et al. 2008; Bouwmeester et al. 2009; Sozer and Kokini 2009; Cushen et al. 2012; Duran and Maezrcato 2013).

Nanotechnology and Nutrient Delivery

Nanostructures in foods can be designed for the targeted delivery of nutrients in the body through highly effective encapsulation and delivery systems. It is useful in applications of food such as delivery systems for vitamins, antimicrobials, antioxidants, flavouring agents, colourants or preservatives (Rizvi et al. 2010). The bioactive compounds such as probiotics and prebiotics have many applications in food nanotechnology (Sozer and Kokini 2009; Kuan et al. 2012). Some of the important applications of nanotechnology in food are water solubility, thermal stability, oral bioavailability, sensory attributes and physiological performance (Huang et al. 2010; Cushen et al. 2012). Whey protein nanospheres (40 nm) can be used as carriers for oral administration of nutraceutical agents to improve their bioavailability (Huang et al. 2010; Kuan et al. 2012).

Microencapsulation Technology

Microencapsulation is a physicochemical or mechanical process in which the bacterial cells are entrapped within coatings of hydrocolloidal materials, which give protection from high acidity and low pH, bile salts and cold shock (Korbekandi et al. 2008; Wenrong and Griffiths 2000; Champagne 2012; Heidebach et al. 2012). Microencapsulation has been used as an efficient method for improving the viability of probiotics in fermented milks and gastrointestinal tract (Iravani et al. 2015).

Encapsulation of bifidobacteria with alginate could significantly increase their viability in frozen ice milk (Iravani et al. 2015). Encapsulated *B. longum* in milk showed higher viability compared with free cells during storage time (Truelstrup Hansen et al. 2002). Higher survivability of *B. infantis* in yoghurt during the refrigerated storage was reported when the cells were encapsulated by a mixture of gellan-xanthan. Report of increase in the viability of lactobacilli in frozen ice milk after encapsulation with alginate has taken place (Sheu and Marshall 1993). Encapsulation process after the encapsulation of *B. infantis* with gellan-xanthan mixture in yoghurt with pH 4 during the 6 weeks of storage period at 4 °C has been reported to have good efficiency (Iravani et al. 2015). Common techniques applied for probiotic microencapsulation are emulsion, extrusion, spray-drying and freeze-drying (Iravani et al. 2015).

Methods of Microencapsulation

There are various techniques used for microencapsulation in the production of probiotic foods. Some of the important and accessible techniques are discussed below.

Extrusion Technique

Extrusion technique, otherwise called as droplet method, is one of the basic techniques of encapsulation of probiotic microorganisms. It is a simple and economical method for good viability of probiotic cells that includes gentle operations and minimal cell injury, as in this technique, the size of beads usually formed from (~2–5 mm) (Iravani et al. 2015) and further, the important disadvantage is slow production of microbeads that it could facilitate large-scale production (Iravani et al. 2015). For microencapsulation of microorganisms, low-temperature extrusion technique is used. In this technique, encapsulation is accomplished in a plasticized composite matrix consisting of fat, flour and starch. The mixture is added to the encapsulated solution, and then the resulting paste (approximately 20% moisture content) is chopped in a chopping system till particles with a diameter range of 0.5–1.5 mm are produced (Mortazavian et al. 2007b; Mortazavian and Sohrabvandi 2006). Production of probiotic fermented tomato juice by Ca-alginate entrapment of *Lb. acidophilus* was also proposed (E King et al. 2007). Similarly, it has also been reported that survival of calcium-induced alginate-starch-encapsulated *Lb. acidophilus* and *B. lactis* was significantly improved when inserted in yoghurt, due to protection of cells (Kailasapathy 2006). In another study, it was reported that microencapsulation in alginates resulted in enhanced resistance of *Lb. casei* in heat processing at temperature 55–65 °C (Mandal et al. 2006), which can be helpful for meat processing.

Emulsion Method

Emulsion technique, otherwise called as two-phase system method, is used for encapsulation of probiotic microorganisms. The emulsion technique is comparatively expensive than the extrusion method, because emulsion formation depends on use of vegetable oil (Mortazavian et al. 2007). Here, cell/polymer slurry (dispersed phase) is added in a lesser amount to the large volume of vegetable oil (continuous phase) such as sunflower, soybean, corn, light paraffin oil, etc. The diameter of beads used is the important factor that significantly influences the metabolic rate, sensory properties and viability of probiotic cells and of the final product. The distribution and dispersion quality of microbeads within the product is also affected (Mortazavian et al. 2007; Mortazavian and Sohrabvandi 2006).

Freeze-Drying

Freeze-drying can be used in microencapsulation of probiotics on a large scale, but the approach exposes the cultures to extreme environmental conditions, and the key parameter is important in providing a high viability level (Anal and Singh 2007). Here, the product is frozen to a temperature below the critical temperature of the formulation. Freeze-drying is a two-phase method; in case of the primary drying, the chamber pressure is lowered, the shelf temperature usually is increased, and the unbound water is removed by sublimation. Then, a secondary drying step is done to remove the bound water by desorption, and lastly the product is brought back to ambient temperature (Jennings 1999; Oetjen 1999). Important factors such as initial cell concentration, pH of the medium, freezing rate, temperature (e.g. the use of liquid nitrogen −196 °C) and content of protective compounds (e.g. carbohydrates) should be controlled in this method (Carvalho et al. 2004). Osmotic shock and membrane injury generally lead to viability losses here resulting from intracellular ice formation and recrystallization (Iravani et al. 2015).

Spray-Drying

The spray-drying technique involves dissolving a polymer, in the continuous phase that surrounds the core material particles inside the sprayed droplets. It is highly helpful for developing water-insoluble dry microcapsule preparations with small and controlled particle size (Picot and Lacroix 2003; Groboillot et al. 1994). The important reasons for using spray-drying are higher stability, easier handling and storage of cultures and limited effects on sensorial and organoleptic properties of foods. Report suggested that Semyonov et al. (2010) evaluated the implementation of spray-freeze-drying (SFD) to produce dry microcapsules of *Lb. paracasei* with high viability. The heat-resistant properties of the strain should be taken into consideration for spray-drying encapsulation of sensitive microorganisms (Mitropoulou et al. 2013).

Immobilization Technique

Immobilization of probiotics has emerged rapidly in food technology. Immobilization refers to the trapping of a material within or throughout a matrix, where the bidirectional diffusion of molecules, like the growth factors, influx of oxygen, nutrients and the outward diffusion of waste products, should be permitted (Mitropoulou et al. 2013). The immobilization technique involves four major categories such as entrapment, adsorption, self-aggregation and mechanical containment based on physical mechanism employed. Cell immobilization provides protection of cell agents against physicochemical changes, such as pH, improved substrate utilization, temperature, higher productivity and efficiency, higher cell densities and cell loads, sterility and faster fermentation and maturation rates (Mitropoulou et al. 2013). Likewise, Reid et al. (2007) investigated the effect of whey protein isolate gel microentrapment on the viability of *Lb. rhamnosus* R011 during the production and storage of biscuits, frozen cranberry juice and vegetable juice (Mitropoulou et al. 2013).

Food Ethics, Safety and Regulations

As functional foods deal with live organisms, their compatibility to the ethical aspect of food and its safety in consumption is of high importance and, hence, regulated by certain bodies. These regulatory bodies have defined certain permissible criteria that the functional food should comply before it is marketed.

Functional Food and Ethics

Human existence fundamentally depends on food, for survival and well-being; further it has spiritual significance and also helps to maintain social relationship. Thus, food bound up with ethics, values and principles of human action. However, global hunger is the major issue in the current world; food ethics has many challenges that emerged with the introduction of innovative technologies for the production of health enhancement food such as “functional food and nutraceuticals” (Schroeder 2007). Do modern technologies produce safe, nutritious and good quality food? Is marketing of food ethically sound? Does the modern production of food have negative effects on the environment like soil erosion, pollution and loss of biodiversity (Mepham 1996)? If at all we have a functional food, what is safe and effective and therefore has the potential to provide an improved state of health and well-being? Two main barriers remain: first, the consumers might not know about its existence and/or functions; second, the consumers might not be able to afford or obtain it.

Safety, Efficacy and Awareness

The efficacy has been established for probiotics in recent years. For example, probiotics added in milk given to children in a day care setting in a randomized trial reduced respiratory infections and severity of illness in them (Hatakka et al. 2001). Diarrhoea was also treated by probiotics in children (Friedrich 2000). This success has attracted the attention of genetic engineers, to "improve" the successful applications and production of genetically modified strain of probiotics, functional foods and nutraceuticals.

Since 1955, Yakult has been on the market with no major safety issues (Heasman and Mellentin 2001). The same product can act differently in two consumers. It can be safe and beneficial for one consumer and might be dangerous for another one. Hence, the safety questions are product or active ingredient specific (Schroeder 2007). Efficacy is generally distinguished from effectiveness in scientific research. The efficacy measures the required effect in stable/ideal conditions, but the effectiveness measures the effect on a large population which is greater under average conditions (Plaami et al. 2001). One major challenge to achieve greater public health effects is poor consumer awareness and knowledge about functional foods. Hence, proper knowledge about health benefits and positive attitude and awareness about functional foods are highly important (Schroeder 2007).

Legislative Problems and Legislative Regulation Around the World

In the USA, there is no Food and Drug Administration (FDA) regulatory policy specific to functional foods; rather "functional foods" are regulated under the same framework as conventional foods. The FDA on December 5, 2006 reported "Improvements needed in overseeing the safety of dietary supplements and 'functional foods" from the General Accounting Office (GAO) (US General Accounting Office 2000). There is as such no formal definition for functional foods. Any food label related to health and nutrition is subjected to regulations. GAO report stated, FDA should "ensure that functional foods provide the functions that they claim and they should give justice to their name" (US General Accounting Office 2000). The major issues of safety, efficacy and human health are the main legislative arguments.

Marketing Approach

The driving force of the global functional food and nutraceutical market is the awareness of consumers towards health benefits of foods and their nutritional benefits for health enhancement and potential disease prevention. Nowadays there are various functional foods in the market throughout the globe (Tables 7.1 and 7.2).

Table 7.1 List of probiotic products available in the market

Brand/trade name	Food type	Sources/strains	Manufacturer company	Country
Aciforce	Freeze-dried product	*Lactococcus lactis, Lactobacillus acidophilus, Enterococcus faecium, Bifidobacterium bifidum*	Biohorma	The Netherlands
Actimel	Probiotic yoghurt drink	*Lactobacillus casei Immunitas*	Danone	France
Activia	Creamy yoghurt	*Bifidus Actiregularis*	Danone	France
Bacilac	Freeze-dried product	*Lactobacillus acidophilus, Lactobacillus rhamnosus*	THT	Belgium
Bactisubtil	Freeze-dried product	*Bacillus sp. strain IP5832*	Synthelabo	Belgium
Bififlor	Freeze-dried product	*Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum*	Eko-Bio	The Netherlands
Hellus	Dairy product	*Lactobacillus fermentum ME-3*	Tallinna Piimatööstuse AS	Estonia
Jovita Probiotisch	Probiotic yoghurt	*Lactobacillus* strain	H & J Bruggen	Germany
Proflora	Freeze-dried product	*Lactobacillus acidophilus, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, Bifidobacterium*	Chefaro	Belgium
Provie	Fruit drink	*Lactobacillus plantarum*	Skanemejerier	Sweden
ProViva	Natural fruit drink and yoghurt	*Lactobacillus plantarum*	Skanemejerier	Sweden
Rela	Yoghurt, cultured milk and juice	*Lactobacillus reuteri*	Ingman Foods	Finland
Revital Active	Yoghurt and yoghurt drink	Probiotics	Olma	Czech Republic
Yakult	Milk drink	*Lactobacillus casei* Shirota	Yakult	Japan
Yosa	Yoghurt-like oat product	*Lactobacillus acidophilus, Bifidobacterium lactis*	Bioferme	Finland
Vifit	Yoghurt drink	*Lactobacillus* strain	Campina	The Netherlands
Vitamel	Dairy products	*Lactobacillus casei GG, Bifidobacterium bifidum, Lactobacillus acidophilus*	Campina	The Netherlands

Source: Siró et al. (2008), Vergari et al. (2010), Kaur and Das (2011)

Table 7.2 Global probiotic food companies

Brand/trade name	Manufacturer company	Country
Aciforce	Biohorma	The Netherlands
Actimel	Danone	France
Activia	Danone	France
Bacilac	THT	Belgium
Bactisubtil	Synthelabo	Belgium
Bififlor	Eko-Bio	The Netherlands
Gefilus	Valio	Finland
Hellus	Tallinna Piimatööstuse AS	Estonia
Jovita Probiotisch	H & J Bruggen	Germany
Proflora	Chefaro	Belgium
Provie	Skanemejerier	Sweden
ProViva	Skanemejerier	Sweden
Rela	Ingman Foods	Finland
Revital Active	Olma	Czech Republic
SOYosa	Bioferme	Finland
SoyTreat	Lifeway	USA
Yakult	Yakult	Japan
Yosa	Bioferme	Finland
Vifit	Campina	The Netherlands
Vitamel	Campina	The Netherlands

Source: Siró et al. (2008), Vergari et al. (2010), Kaur and Das (2011)

Functional Food Market

Globally, acceptance of functional foods and growing awareness among consumers result in good markets in Asia, North America, Western Europe, Latin America, Australia and New Zealand, which are growing constantly with an estimation of about 63 billion US$ by 2010 and expected to reach a value of at least 90.5 billion US$ by 2013 (Kaur and Das 2011). The USA is the largest market of functional food followed by Japan and Europe that together have contributed over 90% of the total sales. There is a rapid increase in the market of functional food in North America with the introduction of a new product such as probiotic into the market (Evani 2009). Canada has experienced a noteworthy growth in this field as evident from the 32% increase in the number of functional food-producing companies, e.g. Harmonium International Inc. (probiotics). Menrad reviewed that more than 1700 functional food products have been launched between 1988 and 1998 (Heasman and Mellentin 2001; Menrad 2003). The growth of the Indian functional food market is estimated at about 12–15% (Kaur and Das 2011). According to a report, India's probiotic market is a "major growth market of the future" with annual growth of 22.6% until 2015. The major backbone for probiotic sales in India is food supplements which account for 49% of sales while foods and beverages at only 4% (Kaur and Das 2011).

Nutraceutical Market Overview

In Asia, Japan is the largest consumer of nutraceuticals. The Japanese nutraceuticals industries produce a variety of products due to the rapid healthy dietary habits among the population (Shimizu 2014). Health consciousness, awareness and willingness to spend on health-fortifying food among people are the main reasons behind the rapidly growing nutraceutical market in India (Keservani et al. 2014). The Indian nutraceutical market has in the past been viewed as an export-focused industry, but with the changing market trends, most local companies have started to launch products in India and expanded their product line according to Indian consumer needs. In 2013, the European nutraceutical market was valued at $6.4 billion and is estimated to grow at an annual rate of 7.2% between 2013 and 2018, to reach a projected $9.0 billion by 2018. According to a report, functional foods remained the fastest-growing segment of the North America nutraceutical market with a 6.5% CAGR during 2007–2011 (M Daliri and Lee 2015). Currently, dietary supplements are the largest segment of nutraceuticals in the USA.

Conclusion

Keeping in view the global demand and consciousness of the prebiotics, probiotics and nutraceuticals, there has been continuous effort in the last decade to meet the demand. In the current chapter, we have discussed the most important developments and inventions in the field such as nanotechnology. Functional foods in the market and status of the products of this segment have also been elucidated. Further, ensuring the quality of such product during commercial scale production is the biggest challenge as live microorganisms are exploited in the production process. Research should focus more on the stability of the products in different climatic zones on the planet. Developed countries should come forward to develop a universal guideline for quality manufacture practice exclusively for probiotics,

Table 7.3 List of prebiotics containing plants

Prebiotics	Sources
A low-molecular-weight polysaccharide	Seaweed
Ulvan	Ulva rigida (green algae)
ß-glucans	*Pleurotus* sp. mushrooms
Inulin-type fructans	Roots of *Morinda officinalis* or Indian mulberry
Oligosaccharide	Yacon root; white- and red-flesh pitayas (dragonfruit)

Source: Pandey et al. (2015)

prebiotics and nutraceuticals. Several prebiotic sources are limited to a particular geographical region; hence the quality manufacture guideline can remove the hindrance of trading of prebiotics, probiotics and nutraceuticals across different countries and continents (Table 7.3).

References

Al-Sheraji SH, Ismail A, Manap MY, Mustafa S, Yusof RM, Hassan FA (2013) Prebiotics as functional foods: a review. J Funct Food 5:1542–1553

Anal AK, Singh H (2007) Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci Technol 18:240–251

Anandharaj M, Sivasankari B, Rani RP (2014) Effects of probiotics, prebiotics, and synbiotics on hypercholesterolemia: a review. Chin J Biol 2014: Article ID 572754

Angioloni A, Collar C (2011) Physicochemical and nutritional properties of reduced-caloric density high fibre breads. LWT Food Sci Technol 44:747–758

Battcock M, Azam-Ali S (2001) Fermented fruits and vegetables: a global perspective. 134:43–69

Berry C (2002) Biologic: functional foods. QJM-Int J Med 95:639–640

Bouwmeester H, Dekkers S, Noordam MY, Hagens WI, Bulder AS, Heer CD, Voorde ECG, Wijnhoven SWP, Marvin HJP, Sips AJAM (2009) Review of health safety aspects of nanotechnologies in food production. Regul Toxicol Pharmacol 53:52–62

Carvalho AS, Silva J, Ho P, Teixeira P, Gibbs P (2004) Relevant factors for the preparation of freeze-dried lactic acid bacteria. Int Dairy J 14:835–847

Cencic A, Chingwaru W (2010) The role of functional foods, nutraceuticals, and food supplements in intestinal health. Forum Nutr 2(6):611–625

Champagne CP (2012) Microencapsulation of probiotics in food: challenges and future prospects. Ther Deliv 3:1249–1251

Corcoran BM, Ross RP, Fitzgerald GF, Stanton C (2004) Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. J Appl Microbiol 96:1024–1039

Criscio TD, Fratianni A, Mignogna R, Cinquanta L, Coppola R, Sorrentino E, Panfili G (2010) Production of functional probiotic, prebiotic, and synbiotic ice creams. J Dairy Sci 93:4555–4564

Cushen M, Morris JKM, Cruz-Romero M, Cummins E (2012) Nanotechnologies in the food industry recent developments, risks and regulation. Trends Food Sci Technol 24:30–46

Daliri EBM, Lee HB (2015) Current trends and future perspectives on functional foods and nutraceuticals. In: Liong M-T (ed) Beneficial microorganisms in food and nutraceuticals, microbiology monographs 27:221–244

Damen B, Pollet A, Dornez E, Broekaert WF, Van Haesendonck I, Trogh I, Arnaut F, Delcour JA, Courtin CM (2012) Xylanasemediated in situ production of arabinoxylan oligosaccharides with prebiotic potential in whole meal breads enriched with arabinoxylan rich materials. Food Chem 131:111–118

De Sousa VMC, dos Santos EF, Sgarbieri VC (2011) The importance of prebiotics in functional foods and clinical practice. Food Nutr Sci 2(2):133–144

De Vrese M, Schrezenmeir J (2008) Probiotics, prebiotics, and synbiotics. In: Food biotechnology. Springer, Berlin, pp 1–66

Döderlein A (1892) Das Scheidensecret und seine Bedeutung f˙ur das Puerperalfieber. Centralblatt f˙ur Bacteriologie 11:699–700

Duran N, Maezrcato PD (2013) Nanobiotechnology perspectives role of nanotechnology in the food industry: a review. Int J Food Sci Technol 48:1127–1134

El Sohaimy SA (2012) Functional foods and nutraceuticals-modern approach to food science. World Appl Sci J 20:691–708

Endo A, Mizuno H, Okada S (2008) Monitoring the bacterial community during fermentation of sunki, an unsalted, fermented vegetable traditional to the Kiso area of Japan. Lett Appl Microbiol 47:221–226

Evani S (2009) Trends in the US functional foods, beverages and ingredients market. Institute of Food Technologists, Agriculture and Agri-Food Canada. pp 1–14

Famularo G, Simone C, Mettuzzi D, Pirovano F (1999) Traditional and high potency probiotic preparations for oral Bacteriotherapy. BioDrugs 12(6):455–470

FAO/WHO (2001) Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria – Joint Food and Agricultural Organization of the United Nations and World Health Organization Expert Consultation Report. C'ordoba, Argentina: http://www.who.int/foodsafety/publications/fsmanagement/probiotics/en/index.html

Figueroa-Gonzalez I, Quijano G, Ramirez G, Cruz-Guerrero A (2011) Probiotics and prebiotics-perspectives and challenges. J Sci Food Agric 91:1341–1348

Friedrich M (2000) A bit of culture for children: probiotics may improve health and fight disease. JAMA 284:1365–1369

Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66:365–378

Gardiner G, O'Sullivan E, Kelly J, Auty MAE, Fitzgerald GF, Collins JK, Ross RP (2000) Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. Appl Environ Microbiol 66:2605–2616

Groboillot AF, Boadi DK, Poncelet D, Neufeld RJ (1994) Immobilization of cells for application in the food industry. CRC Crit Rev Biotechnol 14:75–107

Hatakka K, Savilahti E, Ponka A, Meurman JH, Poussa T, Nase L et al (2001) Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. BMJ 322:1327–1333

Heasman M, Mellentin J (2001) The functional foods revolution. Healthy people. Healthy Earthscan Publications Ltd., London, pp 135–147

Heidebach T, Först P, Kulozik U (2012) Microencapsulation of probiotic cells for food applications. Crit Rev Food Sci Nutr 52:291–311

Huang Q, Yu H, Ru Q (2010) Bioavailability and delivery of nutraceuticals using nanotechnology. J Food Sci 75(1):50–57

Iravani S, Korbekandi H, Mirmohammadi VS (2015) Technology and potential applications of probiotic encapsulation in fermented milk products. J Food Sci Technol 52(8):4679–4696

Jennings TA (1999) Lyophilisation-introduction and basic principles. CRC press, Boca Raton

Kailasapathy K (2006) Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. Food Sci Technol 39(10):1221–1227

Kaur S, Das M (2011) Functional foods: an overview. Food Sci Biotechnol 20(4):861–875

Keller C (2006) Trends in beverages and "Measurable Health". In Proceedings of the third functional food net meeting

Keservani RK, Sharma AK, Ahmad F, Baig ME (2014) Nutraceutical and functional food regulations in India. Food Sci Technol 327–342. https://doi.org/10.1016/B978-0-12-405870-5.00019-0

Khan RU, Naz S (2013) The applications of probiotics in poultry production. Worlds Poult Sci J 69:621–632

King VAE, Y Huang H, H Tsen J (2007) Fermentation of tomato juice by cell immobilized *Lactobacillus acidophilus*. Mid-Taiwan J Med 12(1):1–7

Kingston JJ, Radhika M, Roshini PT, Raksha MA, Murali HS, Batra HV (2010) Molecular characterization of lactic acid bacteria recovered from natural fermentation of beet root and carrot Kanji. Indian J Microbiol 50:292–298

Korbekandi H, Jahadi M, Maracy M, Abedi D, Jalali M (2008) Production and evaluation of a probiotic yogurt using *Lactobacillus casei* ssp. Int J Dairy Technol 62:75–79

Krasaekoopt W, Bhandari B, Deeth H (2003) Evaluation of encapsulation techniques of probiotics for yoghurt. Int Dairy J 13:3–13

Kuan CY, Fung WY, Yuen KH, Liong MT (2012) Nanotech: propensity in foods and bioactives. Crit Rev Food Sci Nutr 52:55–71

Kuo SM (2013) The interplay between fiber and the intestinal microbiome in the inflammatory response. Adv Nutr: Intern Rev J 4(1):16–28

Lilly DM, Stillwell RH (1965) Probiotics growth promoting factors produced by micro-organisms. Science 147:747–748

Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev 4(8):118–126

Mandal S, Puniya AK, Singh K (2006) Effect of alginate concentrations on survival of microencapsulated *Lactobacillus casei* NCDC-298. Int Dairy J 16(10):1190–1195

Martıín R, Miquel S, Ulmer J, Kechaou N, Langalla P, Bermu'dez-Humara'n LG (2013) Role of commensal and probiotic bacteria in human health: a focus on inflammatory bowel disease. Microb Cell Factories 12:71

Menrad K (2003) Market and marketing of functional food in Europe. J Food Eng 56:181–188

Mepham B (1996) Ethical analysis of food biotechnologies: an evaluative framework. In: Mepham B (ed) Food ethics. Routledge, London, pp 101–119

Metchnikoff E (1908) The prolongation of life – optimistic studies. Butterworth-Heinemann, London

Mitropoulou G, Nedovic V, Goyal A, Kourkoutas Y (2013) Immobilization technologies in probiotics food production. J Nutr Metab 2013:1–15

Mitsou EK, Panopoulou N, Turunen K, Spiliotis V, Kyriacou A (2010) Prebiotic potential of barley derived b-glucan at low intake levels: a randomized, double-blinded placebo-controlled clinical study. Food Res Int 43:1086–1092

Mortazavian AM, Sohrabvandi S (2006) Probiotics and food probiotic products; based on dairy probiotic products. Eta Publication, Tehran

Mortazavian AM, Razavi SH, Ehsani MR, Sohrabvandi S (2007) Principles and methods of microencapsulation of probiotic microorganisms. Iran J Biotechnol 5:1–18

Oetjen GW (1999) Freeze-drying. Wiley-VCH, Weinheim

Oliveira RPDS, Florence ACR, Perego P, Oliveira MND, Converti A (2010) Use of lactulose as prebiotic and its influence on the growth, acidification profile and viable counts of different probiotics in fermented skim milk. Int J Microbiol 145:22–27

Palozza PN, Parrone AC, Simone R (2010) Tomato lycopene and inflammatory cascade: basic interactions and clinical implications. Curr Med Chem 17:2547–2563

Panda SK, Behera SK, Qaku XW, Sekar S, Ndinteh DT, Nanjundaswamy HM, Ray RC, Kayitesi E (2017) Quality enhancement of prickly pears (Opuntia sp.) juice through probiotic fermentation using *Lactobacillus fermentum* ATCC 9338. LWT – Food Sci Technol 75:453–459

Pandey RK, Naik RS, Vakil VB (2015) Probiotics, prebiotics and synbiotics- a review. J Food Sci Technol 52(12):7577–7587

Picot A, Lacroix C (2003) Effects of micronization on viability and thermo tolerance of probiotic freeze-dried cultures. Int Dairy J 13:455–462

Plaami SP, Dekker M, van Dokkum W, Ockhuizen T (2001) Functional foods position and future perspectives. NRLO, The Hague. report no 2000/15, 2001

Pokusaeva K, Fitzgerald GF, van Sinderen D (2011) Carbohydrate metabolism in *Bifidobacteria*. Gen Nutr 6(3):285–306

Power DA, Burton JP, Chilcott CN, Dawes PJ, Tagg JR (2008) Preliminary investigations of the colonization of upper respiratory tract tissues of infants using a paediatric formulation of the oral probiotic *Streptococcus salivarius* K12. Eur J Clin Microbiol Infect Dis 27:1261–1263

Reid AA, Champagne CP, Gardner N, Fustier P, Vuillemard JC (2007) Survival in food systems of *Lactobacillus rhamnosus* R011 micro entrapped in whey protein gel particles. J Food Sci 72(1):M031–M037

Rizvi SSH, Moraru, CI, Bouwmeester H, Kampers FWH. (2010). Nanotechnology and food safety. In E. B. Christine, S. Aleksandra, O. Sangsuk, & H. L. M. Lelieveld (Eds.), Ensuring global food safety (pp. 263–280). San Diego, CA: Academic Press

Ro¨ßle C, Brunton N, Gormley RT, Ross PR, Butler F (2010) Development of potentially synbiotic fresh-cut apple slices. J Funct Foods 2:245–254

Sanders ME (2003) Probiotics: considerations for human health. Nutr Rev 61:91–99

Schrezenmeir J, de Vrese M (2001) Probiotics, prebiotics and synbiotics: approaching a definition. Am J Clin Nutr 73:361–364

Schroeder D (2007) Public health, ethics, and functional foods. J Agric Environ Ethics. (2007) 20:247–259

Semyonov D, Ramon O, Kaplun Z, Levin-Brener L, Gurevich N, Shimoni E (2010) Microencapsulation of *Lactobacillus paracasei* by spray freeze drying. Food Res Int 43:193–202

Sheu TY, Marshall RT (1993) Microentrapment of *Lactobacilli* in calcium alginate gels. J Food Sci 58:557–561

Shimizu M (2014) History and current status of functional food regulations in Japan. Food Sci Technol 257–263

Siegrist M, Stampfli N, Kastenholz H, Kelleri C (2008) Perceived risks and perceived benefi ts of different nanotechnology foods and nanotechnology food packaging. Appetite 51:283–290

Sirò I, Kàpolma E, Kàpolma B, Lugasi A (2008) Functional food. Product development, marketing and consumer acceptance-a review. Appetite 51:456–467

Sozer N, Kokini JL (2009) Nanotechnology and its applications in the food sector. Trends Biotechnol 27(2):82–89

Swain MR, Ray RC (2016) Nutritional values and bioactive compounds in fermented fruits and vegetables. In: Paramethioites S (ed) Lactic acid fermentation of fruits and vegetables. CRC Press, Florida, pp 37–52

Swain MR, Anandharaj M, Ray RC, Rani RP (2014) Fermented fruits and vegetables of Asia: a potential source of probiotics. Biotechnol Res Int 2014:250424

Takayuki S, Kazuki K, Fereidoon S (2008) Functional food and health. In the Proceedings of ACS Symposium, p 993

Tammsaar E (2007) Estonian/Baltic functional food market. In Proceedings of the fourth international FFNet meeting on functional foods

Truelstrup Hansen L, Allan-Wojtas PM, Jin YL, Paulson AT (2002) Survival of Ca-alginate microencapsulated *Bifidobacterium* spp. in milk and simulated gastro-intestinalconditions. Food Microbiol 19:35–45

Uchida M, Mogami O, Matsueda (2007) Characteristic of milk whey culture with *Propionibacterium freudenreichii* ET-3 and its application to the inflammatory bowel disease therapy. Inflammopharmacology 15(3):105–108

US General Accounting Office (2000) Report to Congressional Committees. Food safety. Improvements needed in overseeing the safety of dietary supplements and functional foods. http://www.gao.gov/new.items/rc00156.pdf

Vergara CMAC, Honorato TL, Maia GA, Rodrigues S (2010) Prebiotic effect of fermented cashew apple (Anacardium occidentale L) juice. LWT-Food Sci Technol 43:141–145

Vergari F, Tibuzzi A, Basile G (2010) An overview of the functional food market: from marketing issues and commercial players to future demand from life in space. Adv Exp Med Biol 698:308–321

Vujic G, Jajac KA, Despot SV, Kuzmic VV (2013) Efficacy of orally applied probiotic capsules for bacterial vaginosis and other vaginal infections: a double-blind, randomized, placebocontrolled study. Eur J Obstet Gynecol Reprod Biol 168:75–79

Wenrong S, Griffiths M (2000) Survival of bifidobacteria in yogurt and simulated gastric juice following immobilization in gellan-xanthan beads. Int J Food Microbiol 61:17–25
Wildman RE (2001) Handbook of nutraceuticals and functional foods (1st). CRC Series in Modern Nutrition. CRC Press, Boca Raton, USA
Yakult Central Institute for Microbiological Research (1999) Lactobacillus casei Shirota – intestinal flora and human health. Yakult Honsha Co., Ltd., Tokyo
Zhang MM, Cheng JQ, Lu YR, Yi ZH, Yang P, Wu XT (2010) Use of pre-, pro-and synbiotics in patients with acute pancreatitis: a metaanalysis. World J Gastroenterol 16(31):3970. https://doi.org/10.3748/wjg.v16.i31.3970

Chapter 8
Probiotic Dairy Products: Inventions Toward Ultramodern Production

Spiros Paramithiotis and Eleftherios H. Drosinos

Abstract Application of the latest approaches and protocols in probiotic research has resulted in significant advances over the last decade. These refer almost exclusively to the design of probiotic dairy products, mainly through the design of the probiotic culture incorporated. Several protocols have been developed for the assessment of probiotic potential through omic approaches, and many more are currently under development. In addition, through the improvement of our knowledge regarding the mechanisms that lead to infections and disorders, the genetic engineering of probiotic strains aiming at the delivery of bioactive molecules to specific cites was made possible. All these indicate that we are entering an exciting new era with great expectations.

Keywords Probiotics · Starter culture · Selection · Omics · Genetic engineering

Introduction

The probiotic concept is one of the favorites among the researchers due to the width of the topic and the importance for health and well-being. Dairy products are the main vehicle for probiotic delivery worldwide, with several other products considered for this scope including fermented meat products, fruits, and vegetables (Montoro et al. 2016; Park and Jeong 2016; Neffe-Skocinska et al. 2017). A large number of these are already available in the market.

Recent advances, especially in the field of molecular biology, have allowed several improvements to take place. From a production point of view, innovations have occurred in the field of design of a probiotic product. Manufacture of a probiotic product essentially involves three steps: (a) selection of the starter culture, which is

---

S. Paramithiotis (✉) · E. H. Drosinos
Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
e-mail: sdp@aua.gr

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_8

based on the ability to reach a specific niche within the gastrointestinal track of the host, colonize it, and confer the probiotic action; (b) technological evaluation of the starter culture, which is based on the ability of the starter culture to propagate at industrial level and retain viability and functionality after a series of processing steps; and (c) incorporation into the product, a probiotic culture may be incorporated as a starter or an adjunct culture.

Research in recent years has focused on the design of starter cultures. This can take place either by evaluating the potential of wild isolates or by engineering heavily studied strains. In the first case, the genetic background of many desired properties has been identified allowing the evaluation of the probiotic potential to take place through genetic determinants. On the other hand, improvements in the understanding of the mechanisms leading to infections and disorders have allowed the genetic engineering of probiotic strains for the delivery of specific bioactive molecules with prophylactic and/or therapeutic function. In the succeeding sections, recent advances in the field of probiotic product design are presented and critically discussed.

Probiotic Culture Selection

Culture selection involves the assessment of a culture's safety, efficiency to reach the colonization site, and probiotic potential, considering the possible health benefits exerted to the consumer. These functions are currently predicted almost exclusively through in vitro tests. Recent advances in the field of molecular biology have allowed the prediction of these properties through the application of omic approaches.

Safety was traditionally assessed through hemolytic activity, antibiotic resistance, as well as production of enzymes (e.g., hyaluronidase, gelatinase), toxins (e.g., cytolysin), and biogenic amines. In the latter years, safety assessment is usually performed through the detection of the respective genes (e.g., *cylA/B* encoding cytolysins, *hyl* encoding hyaluronidase, *gelE* encoding gelatinase, *tet*(M), *tet*(K), and *tet*(W) responsible for tetracycline resistance, etc.) along with several virulence-associated ones. Especially regarding the latter, detection of *agg* (aggregation protein), *esp* (enterococcal surface protein), *asa1* (aggregation substance), *ace* (collagen protein adhesin), and $efaA_{fs}$ (cell wall adhesin) is more often (Perumal and Venkatesan 2017; Hwanhlem et al. 2017; Motahari et al. 2017; Ojekunle et al. 2017; Guo et al. 2017; Rzepkowska et al. in press).

Efficiency to reach the colonization site is predicted through the ability to survive the stresses faced within the host and colonization potential. In the case of the former, ability to maintain high populations after exposure to pH values ranging from one to three in the presence of pepsin (simulating gastric juice conditions) and alkaline pH values in the presence of bile salts and pancreatin (simulating intestinal juice conditions) is the criterion applied more often. Colonization potential is predicted mostly through the assessment of cell surface hydrophobicity, cellular autoaggregation ability, binding to solubilized collagen, human or animal mucus, and adhesion to various cell lines, mostly Caco-2 and HT29. The disadvantages of in vitro testing were addressed with the use of animal models. However, in that case, other restrictions are introduced, including ethical ones, depending on the type of animal model (Yadav et al. 2017). Although the genome of several lactic acid bacteria species has been described, markers of probiotic features have been detected (Abriouel et al. 2017), and survival mechanisms and strategies to adhere to surfaces have been described (Bove et al. 2012; Arena et al. 2017), no omic approach has been utilized yet as an indicator. However, prediction through in silico models has been performed (Lee et al. 2000).

The desired functional properties of a probiotic culture are constantly updated; they include antagonistic activity against potentially pathogenic microorganisms, particularly invasive Gram-negative pathogens, as well as a series of assets that are beneficial to the host. The latter may either have prophylactic or therapeutic character (Varankovich et al. 2015). A wealth of literature is currently available on that subject and includes both tentative and demonstrated positive effects. In general, the health benefits that have been claimed include modulation of immune responses, protection of the function of the mucosal barrier, reduction of cholesterol levels, anticancer activity, as well as activity against gastrointestinal diseases.

The modulation of immune responses by probiotic cultures refers to the ability of the host to distinguish between the beneficial and pathogenic microorganisms through immune tolerance/hyporesponsiveness and humoral and cell-mediated immune mechanisms, respectively. The mechanisms through which the selective response is activated have been critically presented and discussed by Hardy et al. (2013). The term intestinal or mucosal barrier refers to the physical and immunological barrier that separates the luminal contents and the interstitial tissue and prevents the diffusion of factors that may affect negatively the host (Hardy et al. 2013; Rao and Samak 2013). The epithelial monolayer constitutes the physical barrier, while mucus, protease-resistant IgA, and antimicrobial peptides constitute the immunological. Both barriers are positively affected by the function of probiotics. More accurately, probiotics have been reported to upregulate the expression of the epithelial growth factor (EGF-R) and the pattern recognition receptor (TLR-2) (Resta-Lenert and Barrett 2003; Cario et al. 2004) along with the production of MUC2 and MUC3 intestinal mucins, TGFβ, IL-6 and IL-10 (Rodrigues et al. 2000; Rautava et al. 2006; He et al. 2007; Shang et al. 2008). These functions may be assessed through a variety of phenotypic assays that have been recently reviewed by Papadimitriou et al. (2015).

Interestingly, the interaction with the host may take place even in a probiotic culture viability-independent manner through the activation of responses upon recognition of specific bacterial components or metabolites (Adams 2010). Indeed, inactivated whole cells of *Lactobacillus casei* strain Shirota upregulated IL-12, IL-10, and IL-2 and inhibited the production of IgE, IgG1, IL-4, IL-5, IL-6, and IL-13, while TNFα and TNFγ provided with a mixed response (Matsuzaki and Chin 2000; Cross et al. 2004; Lim et al. 2009). There is currently a wealth of literature on the effect of inactivated whole cells, cell wall components, lipoteichoic acids, and even genomic DNA of a wide range of probiotic and potential probiotic cultures on the immune response of mouse and human cells, cell lines, and macrophages as measured by indicators such as interleukins (such as IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13), factors (such as TGF-β andTNF-α), and immunoglobulins (such as IgA, IgE, and IgG1) (Lammers et al. 2003; Matsuguchi et al. 2003; Shida et al. 2006; Mastrangeli et al. 2009; Kaji et al. 2010; Jensen et al. 2010; van Hoffen et al. 2010). These studies and many more along with a discussion on the possible mode of action have been comprehensively reviewed by Taverniti and Guglielmetti (2011). These functions indicate the efficacy of ghost probiotics and highlight the need for reassessment of the whole probiotic concept, including nomenclature.

Anticancer activity has been attributed to a series of actions including decomposition of carcinogenic compounds, production of compounds with anticarcinogenic activity, inhibition of cancer cell proliferation, and induction of apoptosis as well as modification of the composition and metabolic activity of intestinal microbiota.

Prevention of colorectal cancer (CRC) through probiotics has been extensively studied (dos Reis et al. 2017). Each of the aforementioned parameters plays its own role in the decrease of the colorectal cancer risk. Maintenance of a healthy intestinal microbiota reduces the risk of CRC both directly and through immumodulation. Moreover, reduction of β-glucuronidase and nitrate reductase activities by the intestinal biota has been proposed as a possible mechanism for the reduction of the production of metabolites that have been associated with the development of CRC (Hatakka et al. 2008; Mohania et al. 2013; Verma and Shukla 2013; Zhu et al. 2013). Increase in the production of anti-inflammatory cytokines, with a parallel decrease in the production of pro-inflammatory ones (Koboziev et al. 2013; Zhu et al. 2013) along with the production of short-chain fatty acids and conjugated linoleic acid, as well as compounds with established carcinogenic activity from probiotics or potential probiotic bacteria have been shown to reduce colorectal cancer risk (Ewaschuk et al. 2006; Hosseini et al. 2011; Bassaganya-Riera et al. 2012; Kumar et al. 2012; Vipperla and O'Keefe 2012; Serban 2014).

The use of probiotics has been proved beneficial against a series of gastrointestinal tract (GIT) disorders including infectious, antibiotic-associated, and travelers'diarrhea (Sullivan and Nord 2005; McFarland 2007; Preidis et al. 2011; Girardin and Seidman 2011; Maziade et al. 2013; Patro-Golab et al. 2015; Szajewska and Kolodziej 2015a, b; Lau and Chamberlain 2016), irritable bowel syndrome (O'Mahony et al. 2005; Whorwell et al. 2006; Lorenzo-Zuniga et al. 2014; Yoon et al. 2014), pouchitis (Turroni et al. 2010; Shen et al. 2014; Tomasz et al. 2014), and *H. pylori* infection (Mukai et al. 2002; Tong et al. 2007; Dore et al. 2015; Holz et al. 2015; Szajewska et al. 2015). Specific effects on each of the aforementioned along with the respective mode of action have been recently presented by Domingo (2017).

Cholesterol-reducing capacity of probiotics has been repeatedly exhibited and recently reviewed by Ishimwe et al. (2015). A series of mechanisms have been proposed including enzymatic deconjugation of bile, coprecipitation of cholesterol with deconjugated bile, binding to probiotic cell surface, and conversion to coprostanolin that is excreted in feces (Daliri and Lee 2015).

The above mentioned functions have been studied in depth and known to influence the choice of probiotic cultures. Despite the fact that several mechanisms of action have been described, no omic approach has been yet applied for their prediction.

Technological Evaluation of Probiotic Cultures

Evaluation of a strain's capacity from a technological point of view is very often neglected. However, the ability to reach high population during industrial production scale, capacity to withstand processing such as drying or freezing, and the ability to remain viable and retain functionality during food processing and storage are crucial and strain-dependent properties.

Industrial-scale biomass production takes place in bioreactors whose capacity may reach several hundred liters. Specific attention should be paid to the nutrient content, pH value, dissolved oxygen, and temperature that very often compromise scale-up of biomass production.

Preservation of Probiotic Cultures

Freezing or drying is the processes most commonly applied for the preservation of probiotic cultures. Regarding the former, the rate of temperature decrease is the most crucial factor. High rate creates small ice crystals evenly distributed that minimizes the damage caused by mechanical or osmotic stresses during both freezing and thawing. However, drying is the most commonly process of choice for culture preservation because it may facilitate stability and shelf-life of the culture and on the other hand reduce the logistics costs. Among the drying techniques, spray-drying is most commonly applied in the case of dairy products (Huang et al. 2017). The factors that affect culture viability include the inlet temperature that may be as high as 200 °C (Silva et al. 2002, 2005), the dehydration itself, and the subsequent storage conditions. The strategy most commonly applied to improve viability is the use of appropriate growth conditions before drying and the addition of protective molecules, such as skim milk, polydextrose, inulin, etc. These factors along with many more have been critically discussed by Silva et al. (2011). In general, resistance to the stresses inflicted by spray-drying, namely, heat and osmotic, seems to be a strain-dependent property. However, there are reports stating that *Propionibacterium* spp. are usually more resistant than *Bifidobacterium* spp., *Lactobacillus* spp., and *Lactococcus* spp. (Schuck et al. 2013; Huang et al. 2016), *Streptococcus* spp. than *Lactobacillus* spp. (Bielecka and Majkowska 2000; Kumar and Mishra 2004; Wang et al. 2004), and *Bifidobacterium longum* than *B. infantis* (Lian et al. 2002). Omic approaches for the prediction of tolerance are possible to occur in the near future since a series of genes involved in the adaptation to these conditions, such as the *clp* and *opu* genes, have been described, and their effect on survival is already known (Zotta et al. 2017).

Survival of Probiotic Cultures During Storage

Another aspect that has been extensively studied is the survival of the probiotic cultures during storage of the product in which they have been incorporated as a starter or an adjunct culture. It has been reported that factors such as pH value, concentration of organic acids, type and concentration of other ingredients, and storage temperatures may significantly affect the viability of the probiotic cultures (Donkor et al. 2007). Viability during storage of dairy products has been given much consideration. Enrichment of yoghurt with whey proteins (Marafon et al. 2011), flavoring agents (Vinderola et al. 2002), fruit pulps (Kailasapathy et al. 2008; El-Nagga and Abd El–tawab 2012), cereals (Coda et al. 2012; Zare et al. 2012), lactulose (Oliveira et al. 2011), or inulin (Bozanic et al. 2001; Donkor et al. 2007) either had no negative effect or resulted in the enhancement of the survival of the respective probiotic culture that has been incorporated. However, there are also several reports in the literature that claim the exact opposite, i.e., the decrease of the viability (Ranadheera et al. 2012; Bedani et al. 2014) leading to the conclusion that this property is strain-dependent. The viability during production and storage of a variety of cheeses including Feta (Mazinani et al. 2016), soft goat (Radulovic et al. in press), Italico (Blaiotta et al. 2017), Pecorino Siciliano (Pino et al. 2017), white brined (Liu et al. 2017), Minas (Buriti et al. 2007), and Cheddar (Phillips et al. 2006) has also been assessed confirming that cheese is the best product for probiotic delivery and leading to the basic conclusion that this property is strain-dependent. However, due to the complexity of the microecosystem, and the number of genes involved, an omic approach to predict such a virtue is not expected to occur soon.

Genetic Engineering of Probiotic Strains

An alternative approach to the selection procedure is to provide the probiotic culture with the desired properties through bioengineering. These properties may extend from enhanced tolerance to the GIT or technologically relevant conditions to the improvement of the functionality within the host. The rationale behind the use of bioengineering is to address the limitations of the cultures currently characterized as probiotics or potential probiotics. However, there are certain concerns regarding the use of genetically modified organisms in general that are discussed at the end of this paragraph. In the succeeding paragraphs, the most characteristic studies involving genetic engineering of probiotics or potential probiotics are presented aiming to depict the possibilities offered by genetic engineering.

otsB, the gene encoding for trehalose-6-phosphate phosphatase originating from *Propionibacterium freudenreichii* strain B365, was expressed in *Lactococcus lactis* strain MG1363 in order to provide with trehalose synthesis capacity (Carvalho et al. 2011). Then, strains with the ability to produce trehalose exhibited improved tolerance to acid, cold and heat shocks. On the contrary, no improvement in the viability upon exposure to freeze-drying was observed. However, a nearly 100% viability after freeze-drying was reported for the same strain containing *atsBA* genes originating from *E. coli* DH5a (Termont et al. 2006). In addition, improved tolerance to gastric juice as well as resistance to bile was reported. This enhanced tolerance did not interfere with IL-10 secretion by the same strain (Steidler et al. 2000). *Lc. lactis* strain MG1363 was also used by Bermudez-Humaran et al. (2015) to construct recombinant strains able to secrete cytokines (IL-10 or TGF-β1) and serine protease inhibitors (Elafin or Secretory Leukocyte Protease Inhibitor, SLPI). Then, a DSS-induced murine colitis model (C57BL/6 mice) was used to evaluate the effect after oral administration of the recombinant strains. Significant reduction of the inflammation was observed as an effect of the serine protease inhibitors. On the contrary, only moderate anti-inflammatory effect was recorded when IL-10 or TGF-β1 expressing recombinant strains were administered. In addition, overproduction of Elafin obtained by inactivation of HtrA resulted in enhanced reduction of the inflammation indicating dose dependence.

A recombinant strain based on a *Lb. paracasei* strain able to produce *Listeria* adhesion protein was constructed by Koo et al. (2012) in order to outcompete *Listeria monocytogenes* in adhesion, transepithelial translocation, and cytotoxicity in Caco-2 cells. In addition, several wild-type bacteria with probiotic potential were also examined for the same capacity. The latter failed to prevent *L. monocytogenes* infection. On the contrary, the recombinant strain managed to reduce *L. monocytogenes* translocation by 46% after 24 h and cytotoxicity by 99.8% after 1 h.

Focareta et al. (2006) engineered *E. coli* strain DH5a to express glycosyltransferase genes from *Neisseria gonorrhoeae* and *Campylobacter jejuni*. The aim was to produce a mimic of the ganglioside GM_1 receptor and inactivate in situ the cholera toxin. Indeed, administration of the construct significantly protected 3-day-old Swiss mice against fatal challenge with *Vibrio cholerae*. Another approach was employed by Duan and March (2008, 2010). They constructed a strain based on *E. coli* Nissle 1917 able to express cholera autoinducer 1 and studied the effect on *V. cholerae* colonization and virulence gene expression. Regarding the latter, downregulation of virulence gene expression in Caco-2 cells was reported. Furthermore, pretreatment of 2–3-day-old CD-1 mice with the recombinant strain resulted in 69% reduction of the *V. cholerae* intestinal population after 40 h and 80% reduction of the cholera toxin intestinal binding after 8 h.

Volzing et al. (2013) constructed a recombinant *Lc. lactis* strain able to produce Alyteserin-1a and A3APO, two peptides with antimicrobial activity against both Gram-positive and Gram-negative species. The recombinant strains effectively inhibited growth of *E. coli* and *Salmonella* strains in vitro providing with promising results, necessitating further in situ study. *Lc. lactis* was also used to create a recombinant strain expressing Tcd-AC and Tcd-BC, two fragments of the cytotoxins

A (TdcA) and B (TcdB) produced by *Clostridium difficile* (Guo et al. 2015). Purified fragments or the recombinant strain was orally administered to 5–6-week-old pathogen-free C57BL6 mice that were subsequently challenged with *Cl. difficile*. The vaccinated mice exhibited significantly lower mortalities due to the higher IgG and IgA titers.

The construction of a recombinant *Lb. acidophilus* strain able to produce K99 fimbrial protein was reported by Chu et al. (2005). The strain effectively inhibited binding of enterotoxigenic *E. coli* to intestinal epithelium of pigs exhibiting dose-dependence. Similarly, a recombinant *Lc. lactis* strain able to produce a surface-associated flagellin after induction with nisin for 6 h was reported by Sanchez et al. (2011). The recombinant strain was able to outcompete *E. coli* and *Salmonella enterica* strains to adhesion to mucin-coated polystyrene plates.

Apart from the above mentioned approaches, probiotics have been extensively studied as a vehicle for the targeted delivery of bioactive molecules for prevention and/or treatment of various diseases. Among the most characteristic studies performed so far are the following. Ma et al. (2014) reported the construction of a recombinant *Lc. lactis* strain expressing HSP65 with tandem repeats of P277 that was able to combat the onset of diabetes mellitus type 1. Oral administration of the recombinant strain in non obese diabetic mice resulted in reduced insulitis, improved glucose tolerance, and ultimately prevented hyperglycemia.

Antitumor activity of probiotic bacteria has also been considered to some extend with promising results. The study of Wei et al. (2016) is characteristic of the potential applications. In that study, a *B. longum* strain was engineered to produce tumstatin, an effective angiostatin that inhibits proliferation and induces apoptosis of tumorous endothelial cells. The in situ effectiveness of this approach was examined after intragastric administration of the recombinant strain to tumor-bearing mice. The antitumor effects recorded were significant and very promising for further study.

Finally, Chamcha et al. (2015) reported the construction of a *Lc. lactis*-based strain able to produce HIV-1 Gag-p24 antigen. The aim was to induce HIV-specific immune responses in BALB/c mice and achieve immunity. Indeed, a strong humoral and cellular immunity against HIV was obtained through oral administration of the recombinant strain in which the antigen was expressed on the tip of Group A *Streptococcus* pilus.

The aforementioned approach, although promising, still requires the use of genetically modified organisms (GMOs) for which there are certain concerns. These concerns may result from predicted (Stemke 2004) or unpredicted functions (Hill Jr. et al. 1993). However, there are approaches that may lead to the improvement of the potential probiotic cultures without the need of genetic modification. Such an improvement may occur mainly through directed evolution and dominant selection (Derkx et al. 2014). These approaches may not lead to the development of strains with the improved or targeted health benefits described above, but may result in enhanced resistance to certain stresses and out-competition of pathogens. Such phenotypes may be obtained through adaptation to specific adverse conditions. However, adaptation is only temporary and is still under debate whether it is possible to inflict permanent changes without any change in the genetic material. Epigenetics may provide with a solution, but further study is still necessary to detect and understand the mechanisms involved. Moreover, such an approach requires high level of expertise, time, and effort with safety assessment being still a prerequisite for industrial use.

Conclusions and Future Perspectives

A series of exciting advances in the design of probiotic products have taken place over the last decade. The majority of them refer to the selection of the most appropriate strains, regarding the desired properties or the approach used for their assessment. In parallel, strains with probiotic properties, referring at least to their ability to reach the colonization niche within the host, have been used in genetic engineering studies for targeted delivery of bioactive molecules aiming at the treatment and/or prevention of infections and diseases. The number of such studies is expected to increase within the next few years enriching the selection criteria for the characterization of potential probiotic strains. Moreover, it is very likely that meta-transcriptomic approaches will find their way into the in situ assessment of the GIT microecology and the effect of the probiotic strains.

References

Abriouel H, Perez Montoro B, Casimiro-Soriguer CS et al (2017) Insight into potential probiotic markers predicted in *Lactobacillus pentosus* MP-10 genome sequence. Front Microbiol 8:891

Adams CA (2010) The probiotic paradox: live and dead cells are biological response modifiers. Nutr Res Rev 23:37–46

Arena MP, Capozzi V, Spano G et al (2017) The potential of lactic acid bacteria to colonize biotic and abiotic surfaces and the investigation of their interactions and mechanisms. Appl Microbiol Biotechnol 101:2641–2657

Bassaganya-Riera J, Viladomiu M, Pedragosa M, Simone C, Hontecillas R (2012) Immunoregulatory mechanisms underlying prevention of colitis-associated colorectal cancer by probiotic bacteria. PLoS ONE 7:1–8

Bedani R, Vieira ADS, Rossi EA et al (2014) Tropical fruit pulps decreased probiotic survival to in vitro gastrointestinal stress in symbiotic soy yoghurt with okara during storage. LWT-Food Sci Technol 55:436–443

Bermudez-Humaran LG, Motta J-P, Aubry C et al (2015) Serine protease inhibitors protect better than IL-10 and TGF-β anti-inflammatory cytokines against mouse colitis when delivered by recombinant lactococci. Microb Cell Factories 14:26

Bielecka M, Majkowska A (2000) Effect of spray drying temperature of yoghurt on the survival of starter cultures, moisture content and sensoric properties of yoghurt powder. Nahrung/Food 44:257–260

Blaiotta G, Murru N, Di Cerbo A et al (2017) Commercially standardized process for probiotic "Italico" cheese production. LWT – Food Sci Technol 79:601–608

Bove P, Fiocco D, Gallone A et al (2012) Abiotic stress responses in lactic acid bacteria. In: Wong HC (ed) Stress response of foodborne microorganisms. Nova Publishers, New York, pp 355–403

Bozanic R, Rogelj I, Tratni IJ (2001) Fermented acidophilus goat's milk supplemented with inulin: comparison with cow's milk. Milchwissenschaft 56:618–622
Buriti FCA, Okazaki TY, Alegro JHA et al (2007) Effect of a probiotic mixed culture on texture profile and sensory performance of Minas fresh-cheeses in comparison with the traditional products. Arch Latinoam Nutr 57:179–185
Cario E, Gerken G, Podolsky DK (2004) Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C. Gastroenterology 127:224–238
Carvalho AL, Cardoso FS, Bohn A (2011) Engineering trehalose synthesis in *Lactococcus lactis* for improved stress tolerance. Appl Environ Microbiol 77:4189–4199
Chamcha V, Jones A, Quigley BR et al (2015) Oral immunization with a recombinant *Lactococcus lactis*–expressing HIV-1 antigen on group A *Streptococcus* pilus induces strong mucosal immunity in the gut. J Immunol 195:5025–5034
Chu H, Kang S, Ha S et al (2005) *Lactobacillus acidophilus* expressing recombinant K99 adhesive fimbriae has an inhibitory effect on adhesion of enterotoxigenic *Escherichia coli*. Microbiol Immunol 49:941–948
Coda R, Laner A, Trani A et al (2012) Yogurt-like beverages made of a mixture of cereals, soy and grape must: microbiology, texture, nutritional and sensory properties. Int J Food Microbiol 155:120–127
Cross ML, Ganner A, Teilab D et al (2004) Patterns of cytokine induction by gram-positive and gram-negative probiotic bacteria. FEMS Immunol Med Microbiol 42:173–180
Daliri EB-M, Lee BH (2015) New perspectives on probiotics in health and disease. Food Sci Human Wellness 4:56–65
Derkx PMF, Janzen T, Sorensen KI et al (2014) The art of strain improvement of industrial lactic acid bacteria without the use of recombinant DNA technology. Microb Cell Factories 13:S5
Domingo JJS (2017) Review of the role of probiotics in gastrointestinal diseases in adults. Gastroenterol Hepatol 40:417–429
Donkor ON, Tsangalis D, Shah NP (2007) Viability of probiotic bacteria and concentrations of organic acids in commercial yoghurts during refrigerated storage. Food Aust 59:121–126
Dore MP, Goni E, di Mario F (2015) Is there a role for probiotics in *Helicobacter pylori* therapy? Gastroenterol Clin N Am 44:565–575
dos Reis SA, da Conceicao LL, Siqueira NP et al (2017) Review of the mechanisms of probiotic actions in the prevention of colorectal cancer. Nutr Res 37:1–19
Duan F, March JC (2008) Interrupting *Vibrio cholerae* infection of human epithelial cells with engineered commensal bacterial signaling. Biotechnol Bioeng 101:128–134
Duan F, March JC (2010) Engineered bacterial communication prevents *Vibrio cholerae* virulence in an infant mouse model. PNAS 107:11260–11264
El-Nagga EA, Abd El–tawab YA (2012) Compositional characteristics of date syrup extracted by different methods in some fermented dairy products. Ann Agric Sci 57:29–36
Ewaschuk JB, Walker JW, Diaz H et al (2006) Bioproduction of conjugated linoleic acid by probiotic bacteria occurs in vitro and in vivo in mice. J Nutr 136:1483–1487
Focareta A, Paton JC, Morona R et al (2006) A recombinant probiotic for treatment and prevention of cholera. Gastroenterology 130:1688–1695
Girardin M, Seidman EG (2011) Indications for the use of probiotics in gastrointestinal diseases. Dig Dis 29:574–587
Guo S, Yan W, McDonough SP et al (2015) The recombinant *Lactococcus lactis* oral vaccine induces protection against *C. difficile* spore challenge in a mouse model. Vaccine 33:1586–1595
Guo H, Pan L, Li L et al (2017) Characterization of antibiotic resistance genes from *Lactobacillus* isolated from traditional dairy products. J Food Sci 82:724–730
Hardy H, Harris J, Lyon E et al (2013) Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. Forum Nutr 5:1869–1912
Hatakka K, Holma R, El-Nezami H et al (2008) The influence of *Lactobacillus rhamnosus* LC705 together with *Propionibacterium freudenreichii* ssp. *shermanii* JS on potentially carcinogenic bacterial activity in human colon. Int J Food Microbiol 128:406–410

He B, Xu W, Santini PA et al (2007) Intestinal bacteria trigger T cell-independent immunoglobulin A2 class switching by inducing epithelial-cell secretion of the cytokine APRIL. Immunology 26:812–826

Hill RH Jr, Caudill SP, Philen RM et al (1993) Contaminants in L-tryptophan associated with eosinophilia myalgia syndrome. Arch Environ Contam Toxicol 25:134–142

Holz C, Busjahn A, Mehling H et al (2015) Significant reduction in *Helicobacter pylori* load in humans with non-viable *Lactobacillus reuteri* DSM17648: a pilot study. Probiotics Antimicrob Proteins 7:91–100

Hosseini E, Grootaert C, Verstraete W et al (2011) Propionate as a health-promoting microbial metabolite in the human gut. Nutr Rev 69:245–258

Huang S, Cauty C, Dolivet A et al (2016) Double use of highly concentrated sweet whey to improve the biomass production and viability of spray-dried probiotic bacteria. J Funct Foods 23:453–463

Huang S, Vignolles M-L, Chen XD et al (2017) Spray drying of probiotics and other food-grade bacteria: a review. Trends Food Sci Technol 63:1–17

Hwanhlem N, Ivanova T, Biscola V et al (2017) Bacteriocin producing *Enterococcus faecalis* isolated from chicken gastrointestinal tract originating from Phitsanulok, Thailand: isolation, screening, safety evaluation and probiotic properties. Food Control 78:187–195

Ishimwe N, Daliri E, Lee B et al (2015) The perspective on cholesterol-lowering mechanisms of probiotics. Mol Nutr Food Res 59:94–105

Jensen GS, Benson KF, Carter SG et al (2010) GanedenBC30 cell wall and metabolites: anti-inflammatory and immune modulating effects in vitro. BMC Immunol 11:1–15

Kailasapathy K, Harmstorf I, Phillips M (2008) Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* in stirred fruit yogurts. LWT-Food Sci Technol 41:1317–1322

Kaji R, Kiyoshima-Shibata J, Nagaoka M et al (2010) Bacterial teichoic acids reverse predominant IL-12 production induced by certain lactobacillus strains into predominant IL-10 production via TLR2-dependent ERK activation in macrophages. J Immunol 184:3505–3513

Koboziev I, Webb CR, Furr KL et al (2013) Role of the enteric microbiota in intestinal homeostasis and inflammation. Free Radic Biol Med 68:122–133

Koo OK, Amalaradjou MAR, Bhunia AK (2012) Recombinant probiotic expressing Listeria adhesion protein attenuates *Listeria monocytogenes* virulence in vitro. PLoS ONE 7:e29277

Kumar P, Mishra HN (2004) Yoghurt powder-a review of process technology, storage and utilization. Food Bioprod Process 82:133–142

Kumar M, Nagpal R, Verma V et al (2012) Probiotic metabolites as epigenetic targets in the prevention of colon cancer. Nutr Rev 71:23–34

Lammers KM, Brigidi P, Vitali B et al (2003) Immunomodulatory effects of probiotic bacteria DNA: IL-1 and IL-10 response in human peripheral blood mononuclear cells. FEMS Immunol Med Microbiol 38:165–172

Lau CS, Chamberlain RS (2016) Probiotics are effective at preventing *Clostridium difficile*-associated diarrhea: a systematic review and meta-analysis. Int J Gen Med 9:27–37

Lee YK, Lim CY, Teng WL et al (2000) Quantitative approach in the study of adhesion of lactic acid bacteria to intestinal cells and their competition with enterobacteria. Appl Environ Microbiol 66:3692–3697

Lian WC, Hsiao HC, Chou CC (2002) Survival of bifidobacteria after spray drying. Int J Food Microbiol 74:79–86

Lim LH, Li HY, Huang CH et al (2009) The effects of heat-killed wild-type *Lactobacillus casei* Shirota on allergic immune responses in an allergy mouse model. Int Arch Allergy Immunol 148:297–304

Liu L, Li X, Zhu Y et al (2017) Effect of microencapsulation with the Maillard reaction products of whey proteins and isomaltooligosaccharide on the survival rate of *Lactobacillus rhamnosus* in white brined cheese. Food Control 79:44–49

Lorenzo-Zuniga V, Llop E, Suarez C et al (2014) I.31, a new combination of probiotics, improves irritable bowel syndrome-related quality of life. World J Gastroenterol 20:8709–8716
Ma Y, Liu J, Hou J et al (2014) Oral administration of recombinant *Lactococcus lactis* expressing HSP65 and tandemly repeated P277 reduces the incidence of type I diabetes in non-obese diabetic mice. PLoS ONE 9(8):e105701
Marafon AP, Sumi A, Alcantara MR et al (2011) Optimization of the rheological properties of probiotic yoghurts supplemented with milk proteins. LWT-Food Sci Technol 44:511–519
Mastrangeli G, Corinti S, Butteroni C et al (2009) Effects of live and inactivated VSL#3 probiotic preparations in the modulation of in vitro and in vivo allergen-induced Th2 responses. Int Arch Allergy Immunol 150:133–143
Matsuguchi T, Takagi A, Matsuzaki T et al (2003) Lipoteichoic acids from *Lactobacillus* strains elicit strong tumor necrosis factor alpha inducing activities in macrophages through toll-like receptor 2. Clin Diagn Lab Immunol 10:259–266
Matsuzaki T, Chin J (2000) Modulating immune responses with probiotic bacteria. Immunol Cell Biol 78:67–73
Maziade PJ, Andriessen JA, Pereira P et al (2013) Impact of adding prophylactic probiotics to a bundle of standard preventative measures for *Clostridium difficile* infections: enhanced and sustained decrease in the incidence and severity of infection at a community hospital. Curr Med Res Opin 29:1341–1347
Mazinani S, Fadaei V, Khosravi-Darani K (2016) Impact of *Spirulina platensis* on physicochemical properties and viability of *Lactobacillus acidophilus* of probiotic UF feta cheese. J Food Process Preserv 40:1318–1324
McFarland LV (2007) Meta-analysis of probiotics for the prevention of traveler's diarrhea. Travel Med Infect Dis 5:97–105
Mohania D, Kansal VK, Sagwal R et al (2013) Anticarcinogenic effect of probiotic Dahi and piroxicam on DMH-induced colorectal carcinogenesis in Wistar rats. Am J Cancer Ther Pharmacol 1:1–17
Montoro BP, Benomar N, Lerma LL et al (2016) Fermented Alorena table olives as a source of potential probiotic *Lactobacillus pentosus* strains. Front Microbiol 7:1583
Motahari P, Mirdamadi S, Kianirad M (2017) Safety evaluation and antimicrobial properties of *Lactobacillus pentosus* 22C isolated from traditional yogurt. Food Measure 11:972–978
Mukai T, Asasaka T, Sato E et al (2002) Inhibition of binding of *Helicobacter pylori* to the glycolipid receptors by probiotic *Lactobacillus reuteri*. FEMS Immunol Med Microbiol 32:105–110
Neffe-Skocinska K, Okon A, Kolozyn-Krajewska et al (2017) Amino acid profile and sensory characteristics of dry fermented pork loins produced with a mixture of probiotic starter cultures. J Sci Food Agric 97:2953–2960
O'Mahony L, McCarthy J, Kelly P et al (2005) *Lactobacillus* and *Bifidobacterium* in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology 128:541–551
Ojekunle O, Banwo K, Sanni AI (2017) In vitro and in vivo evaluation of *Weissella cibaria* and *Lactobacillus plantarum* for their protective effect against cadmium and lead toxicities. Lett Appl Microbiol 64:379–385
Oliveira RPS, Florence ACR, Perego P et al (2011) Use of lactulose as prebiotic and its influence on the growth, acidification profile and viable counts of different probiotics in fermented skim milk. Int J Food Microbiol 145:22–27
Papadimitriou K, Zoumpopoulou G, Foligne B et al (2015) Discovering probiotic microorganisms: in vitro, in vivo, genetic and omics approaches. Front Microbiol 6:58
Park K-Y, Jeong J-K (2016) Kimchi (Korean fermented vegetables) as a probiotic food. In: Watson RR, Preedy VR (eds) Probiotic, prebiotics and synbiotics. Bioactive foods in health promotion. Academic, London, pp 391–408
Patro-Golab B, Shamir R, Szajewska H (2015) Yogurt for treating antibiotic-associated diarrhea: systematic review and meta-analysis. Nutrition 31:796–800

Perumal V, Venkatesan A (2017) Antimicrobial, cytotoxic effect and purification of bacteriocin from vancomycin susceptible *Enterococcus faecalis* and its safety evaluation for probiotization LWT – Food Sci Technol 78:303–310

Phillips M, Kailasapathy K, Tran L (2006) Viability of commercial probiotic cultures (*L. acidophilus*, *Bifidobacterium* sp., *L. casei*, *L. paracasei* and *L. rhamnosus*) in cheddar cheese. Int J Food Microbiol 108:276–280

Pino A, Van Hoorde K, Pitino I et al (2017) Survival of potential probiotic lactobacilli used as adjunct cultures on Pecorino Siciliano cheese ripening and passage through the gastrointestinal tract of healthy volunteers. Int J Food Microbiol 252:42–52

Preidis GA, Hill C, Guerrant RL et al (2011) Probiotics, enteric and diarrheal diseases, and global health. Gastroenterology 140:8–14

Radulovic Z, Miocinovic J, Mirkovic N et al (in press) Survival of spray-dried and free-cells of potential probiotic *Lactobacillus plantarum* 564 in soft goat cheese. Anim Sci J. https://doi.org/10.1111/asj.12802

Ranadheera CS, Evans CA, Adams MC et al (2012) In vitro analysis of gastrointestinal tolerance and intestinal cell adhesion of probiotics in goat's milk ice cream and yogurt. Food Res Int 49:619–625

Rao RK, Samak G (2013) Protection and restitution of gut barrier by probiotics: nutritional and clinical implications. Curr Nutr Food Sci 9:99–107

Rautava S, Arvilommi H, Isolaur E (2006) Specific probiotics in enhancing maturation of IgA responses in formula-fed infants. Pediatr Res 60:221–224

Resta-Lenert S, Barrett KE (2003) Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). Gut 52:988–997

Rodrigues AC, Cara DC, Fretez SH et al (2000) *Saccharomyces boulardii* stimulates sIgA production and the phagocytic system of gnotobiotic mice. J Appl Microbiol 89:404–414

Rzepkowska A, Zielińska D, Ołdak A et al (in press) Safety assessment and antimicrobial properties of the lactic acid bacteria strains isolated from polish raw fermented meat products. Int J Food Prop in press https://doi.org/10.1080/10942912.2016.1250098

Sanchez B, Lopez P, Gonzalez-Rodrıguez I et al (2011) A flagellin-producing *Lactococcus* strain: interactions with mucin and enteropathogens. FEMS Microbiol Lett 318:101–107

Schuck P, Dolivet A, Mejean S et al (2013) Spray drying of dairy bacteria: new opportunities to improve the viability of bacteria powders. Int Dairy J 31:12–17

Serban DE (2014) Gastrointestinal cancers: influence of gut microbiota, probiotics and prebiotics. Cancer Lett 345:258–270

Shang L, Fukata M, Thirunarayanan N et al (2008) Toll-like receptor signaling in small intestinal epithelium promotes B-cell recruitment and IgA production in lamina propria. Gastroenterology 135:529–538

Shen J, Zuo ZX, Mao AP (2014) Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. Inflamm Bowel Dis 20:21–35

Shida K, Kiyoshima-Shibata J, Nagaoka M et al (2006) Induction of interleukin-12 by lactobacillus strains having a rigid cell wall resistant to intracellular digestion. J Dairy Sci 89:3306–3317

Silva J, Carvalho AS, Teixeira P et al (2002) Bacteriocin production by spray-dried lactic acid bacteria. Lett Appl Microbiol 34:77–81

Silva J, Carvalho AS, Ferreira R et al (2005) Effect of the pH of growth on the survival of *Lactobacillus delbrueckii* subsp. *bulgaricus* to stress conditions during spray-drying. J Appl Microbiol 98:775–782

Silva J, Freixo R, Gibbs P et al (2011) Spray-drying for the production of dried cultures. Int J Dairy Technol 64:321–335

Steidler L, Hans W, Schotte L et al (2000) Treatment of murine colitis by *Lactococcus lactis* secreting Interleukin-10. Science 289:1352–1355

Stemke DJ (2004) Geneticallymodified microorganisms biosafety and ethical issues. In: Parekh SR (ed) The GMO handbook. Genetically modified animals, microbes, and plants in biotechnology. Humana Press, Totowa, pp 85–132

Sullivan A, Nord CE (2005) Probiotics and gastrointestinal diseases. J Intern Med 257:78–92

Szajewska H, Kolodziej M (2015a) Systematic review with meta-analysis: *Lactobacillus rhamnosus* GG in the prevention of antibiotic-associated diarrhoea in children and adults. Aliment Pharmacol Ther 42:1149–1157

Szajewska H, Kolodziej M (2015b) Systematic review with meta-analysis: *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea. Aliment Pharmacol Ther 42:793–801

Szajewska H, Horvath A, Kolodziej M (2015) Systematic review with meta-analysis: *Saccharomyces boulardii* supplementation and eradication of *Helicobacter pylori* infection. Aliment Pharmacol Ther 41:1237–1245

Taverniti V, Guglielmetti S (2011) The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). Genes Nutr 6:261–274

Termont S, Vandenbroucke K, Iserentant D et al (2006) Intracellular accumulation of trehalose protects *Lactococcus lactis* from freeze-drying damage and bile toxicity and increases gastric acid resistance. Appl Environ Microbiol 72:7694–7700

Tomasz B, Zoran S, Jaroslaw W et al (2014) Long-term use of probiotics *Lactobacillus* and *Bifidobacterium* has a prophylactic effect on the occurrence and severity of pouchitis: a randomized prospective study. Biomed Res Int 2014:208064

Tong JL, Ran ZH, Shen J et al (2007) Meta-analysis: the effect of supplementation with probiotics on eradication rates and adverse events during *Helicobacter pylori* eradication therapy. Aliment Pharmacol Ther 25:155–168

Turroni S, Vitali B, Candela M et al (2010) Antibiotics and probiotics in chronic pouchitis: a comparative proteomic approach. World J Gastroenterol 16:30–41

van Hoffen E, Korthagen NM, de Kivit S et al (2010) Exposure of intestinal epithelial cells to UV-killed *Lactobacillus* GG but not *Bifidobacterium breve* enhances the effector immune response in vitro. Int Arch Allergy Immunol 152:159–168

Varankovich NV, Nickerson MT, Korber DR (2015) Probiotic-based strategies for therapeutic and prophylactic use against multiple gastrointestinal diseases. Front Microbiol 6:685

Verma A, Shukla G (2013) Probiotics *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* suppresses DMH-induced procarcinogenic fecal enzymes and preneoplastic aberrant crypt foci in early colon carcinogenesis in Sprague Dawley rats. Nutr Cancer 65:84–91

Vinderola CG, Costa GA, Regenhardt S et al (2002) Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria. Int Dairy J 12:579–589

Vipperla K, O'Keefe SJ (2012) The microbiota and its metabolites in colonic mucosal health and cancer risk. Nutr Clin Pract 27:624–635

Volzing K, Borrero J, Sadowsky MJ et al (2013) Antimicrobial peptides targeting gram-negative pathogens, produced and delivered by lactic acid bacteria. ACS Synth Biol 2:643–650

Wang YC, Yu RC, Chou CC (2004) Viability of lactic acid bacteria and bifidobacteria in fermented soymilk after drying, subsequent rehydration and storage. Int J Food Microbiol 93:209–217

Wei C, Xun AY, Wei XX et al (2016) Bifidobacteria expressing tumstatin protein for antitumor therapy in tumor-bearing mice. Technol Cancer Res Treat 15:498–508

Whorwell PJ, Altringer L, Morel J et al (2006) Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. Am J Gastroenterol 101:1581–1590

Yadav AK, Tyagi A, Kumar A et al (2017) Adhesion of lactobacilli and their anti-infectivity potential. Crit Rev Food Sci Nutr 57:2042–2056

Yoon JS, Sohn W, Lee OY et al (2014) Effect of multispecies probiotics on irritable bowel syndrome: a randomized, double-blind, placebo-controlled trial. J Gastroenterol Hepatol 29:52–59

Zare F, Champagne CP, Simpson BK et al (2012) Effect of the addition of pulse ingredients to milk on acid production by probiotic and yoghurt starter cultures. LWT-Food Sci Technol 45:155–160

Zhu Q, Gao R, Wu W et al (2013) The role of gut microbiota in the pathogenesis of colorectal cancer. Tumor Biol 34:1285–1300

Zotta T, Parente E, Ricciardi A (2017) Aerobic metabolism in the genus *Lactobacillus*: impact on stress response and potential applications in the food industry. J Appl Microbiol 122:857–869

Chapter 9
Non-dairy Probiotic Foods: Innovations and Market Trends

Gargi Dey

Abstract The existence of an enormous list of traditional fermented foods produced and consumed globally may be considered as the genesis of non-dairy probiotic products. While LABs used as starter cultures showed attributes desirable for a probiotic culture, it was just a matter of time, when scientists and technologists figured out that food matrices which could be fermented could also be used as probiotic delivery vehicles and some starter cultures could perform the dual task of fermentation and probiosis. Meanwhile health has continued to be at the forefront of new product development strategies. The observations that plant carbohydrates and phenolics may act synergistically with probiotics in formulations for gut health have given a shot in the arm for non-dairy probiotic product developers. Today, diverse food innovations are possible through manipulations of synergies between probiotic strain and the components of food matrices, although technological bottlenecks needed to be addressed. However, it is expected that new non-dairy product development will pose its own set of challenges. Each food matrix is unique; the industries need to standardize and optimize the basic formulation for each product that will have the required sensory and physical chemical characteristics, extended shelf life, and chemical stability, all at a reasonable cost. Much has been said about technological complexities and challenges of non-dairy food formats, but despite them, several commercially viable products are already gracing the shelves of supermarkets globally. Food formats like fruit and vegetables juices and cut and whole tissue and cereals seem to be the current favorite, while meat matrix is still in the research stage. The key to success will be to convince consumers to pay the cost for a new product, and it can be achieved through communication of unambiguous and truthful health claims to the consumers.

Keywords Non-dairy · Probiotic · Fruit juices · Food matrices · Sea buckthorn

G. Dey (✉)
School of Biotechnology Kalinga Institute of Industrial Technology, Bhubaneswar, India
e-mail: gargi.dey@kiitbiotech.ac.in

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_9

Introduction

This is an age of prevailing lifestyle diseases which is threatening the wellness of the society, age, stress, and low-quality diet being the common reasons behind it. In this scenario, functional foods promise deliverance and prevention from various lifestyle diseases. The focus of new product development program has also shifted to tertiary functions of food which is the disease preventing ability of the food. Meanwhile, innovative and progressive food companies have seized this opportunity arising from consumer interest in functional foods. Over the years functional foods became more directed toward food additives that may exert a positive effect on the gut microbiota composition; the focus has largely been on probiotics, prebiotics, and more recently synbiotics.

Since, historically, yogurt was known to restore the gut microflora, understandably, the first-generation probiotic food products were all dairy-based. However, the companies involved in product development soon realized, from consumer behavior analysis, that they needed a proactive as opposed to a reactive approach to novel product development especially in case of probiotics. Soon the supermarket shelves of Asia, Europe, and the USA started to carry a small range of non-dairy probiotic items. Today, almost every food group, be it cereals, soy, fruits and vegetables, or meat, has been inspected, explored, and evaluated with regard to its suitability as a viable and shelf-stable probiotic carrier.

This chapter gives an overview of the advantages and constraints of each conventional and unconventional non-dairy food matrix and the latest commercial products available globally. The projected market fate of this diverse, continuously evolving, food segment has also been discussed.

Product Development

It has been stated that the strongest drivers of non-dairy products are vegetarianism, milk cholesterol content, lactose intolerance, and the consumer interest in shelf diversity and sensory appeal. From the industry viewpoint, many manufacturers are seeking ways to create and increase value, which has further resulted in an increase in product profile. However, a more compelling reason and stronger driver have been the emerging evidences of health benefits that can be acquired through symbiotic relationship between plant components and probiotics and gut commensals (Duda-Chodak et al. 2015; Valdes et al. 2015; Ozdal et al. 2016).

The existence of an enormous list of traditional fermented foods produced and consumed globally should be considered as the genesis of non-dairy probiotic products. The rationale of non-dairy probiotic was borrowed from traditional fermented foods, because it became evident that allochthonous or autochthonous lactic acid bacteria (LABs) possessed gastric and bile tolerance, have the capacity to adhere to gut epithelial cell lines (Caco-2), metabolize host prebiotics, incite antagonistic activity toward pathogens and spoilage organisms, and elicit immunomodulatory activities (Vitalli et al. 2012; Di Cagno et al. 2013). In short, some of the LABs used as starter cultures showed attributes desirable for a probiotic culture. Some of the autochthonous strains even showed cholesterol-reducing abilities (Lee et al. 2011). Hence, it was just a matter of time, when scientists and technologists figured out that those food matrices which could be fermented could also be used as probiotic delivery vehicles and some starter cultures could perform the dual task of fermentation and probiosis.

Approaches to Product Development

Food innovations happen either due to consumer demand or due to advances in science and technology. A successful approach to probiotic product development should address the following: (a) market trends and consumer preferences, (b) the physicochemical properties of the selected food matrix, and (c) the potential interaction between the probiotic strain and the food ingredients of the matrix during processing and storage period.

Health will continue to be at the forefront of new product development strategies. Food and beverage manufacturers are looking to incorporate probiotic into all types of food groups and matrices. Diverse food innovations are possible through manipulations of synergies between probiotic strain and the component present in the food matrices. However, there are several concerns and issues that have been raised regarding the probiotic product development. Health claim credibility, market access requirements, and lack of product awareness among consumers have proven to be the road blocks in the further commercial expansion of this food category. For better customer acceptance, novel product developers need to make them in formats that the customers are familiar with.

Non-dairy Food Matrices

The observations that plant compounds, like complex carbohydrates and phenolics, may act synergistically with probiotics (Pupponen et al. 2002; Selma et al. 2009) in formulations for gut health have given a shot in the arm for non-dairy probiotic product developers. Every food category from cereals to soy, to fruits and vegetables, and finally to meat has been the subject of research for new product development. As expected the new non-dairy product development comes with its own set of challenges. Each food matrix is unique, so the industries need to standardize and optimize the basic formulation for each product that will have the required sensory and physical chemical characteristics, extended shelf life, and chemical stability, all at a reasonable cost. Critical factors to be considered are pH, ionic strength, macro- and microstructure, water activity, oxygen levels, presence of competing microorganisms, and inhibitors in the food matrices which can directly influence the probiotic survival. For example, cereal- and fruit-based matrices are usually acidic and necessitate protection of probiotic through supplementation or microencapsulation. Another equally important consideration is the storage conditions. Very often it is seen that beverages and cereal desserts which are usually stored at room temperature can drastically change either the probiotic survival or the sensory attributes of the product (Matilla-Sandholm et al. 2002). Nevertheless, several technologies and shelf-stable non-dairy products have evolved in the past decades. Among non-dairy matrices, soy-based probiotic beverages have been popular and have been discussed extensively by Granto et al. 2010. It must be pointed out that products created from soy resemble more or less dairy products like yogurts. In this chapter, the focus is more on different product designs which do not resemble their dairy counterparts; hence in the subsequent sections, fruit and vegetable, cereal, and meat matrices have been discussed in detail.

Fruit and Vegetable Matrices

Interesting information was reported by Rahavi and Kapsak (2010), that some of the functional food categories as ranked by customers were as follows: fruits and vegetables; fish/seafood; dairy; meat and poultry; tea, green tea; whole grains; and finally cereals.

Fruits and vegetables are consumed because of the perceived health benefits due to the presence of antioxidant compounds like anthocyanin, flavanols, epicatechins, flavanones, procyanidins, lignans, carotenoids, soluble and insoluble fiber, isothiocyanates, phenolic acids, and sulfides along with vitamins C and E. Food manufacturers have capitalized on this “healthy” and “good for you” image of fruits and vegetables. Recent trend has emerged where fruit and vegetable juices are further being infused with nutraceuticals and probiotic strains as a means of value addition.

In case of some fruits and vegetables as a non-dairy probiotic carrier, one must be cautious that sometimes their higher polyphenolic, organic acids or dietary fibers reduce their sensory acceptability. For example, the juice of sea buckthorn berries (*Hippophae rhamnoides*) is known for its high phenolic acid, ascorbic acid, and fatty acid content (Negi and Dey 2009) which gives it a tart taste with low palatability. To tackle this problem, a tailored formulation was designed in our lab to give a shelf-stable probiotic-fortified sea buckthorn beverage (Sireswar et al. 2017a). The successful transformation of the fruit-based matrices into physiologically functional food depends on targeted interactions between probiotics, natural or added prebiotics, and other food components during the various unit operations of food processing. It is possible to increase the efficacy of the product by designing food matrix with synergistic or additive interactions between probiotic strain and ingredients. For instance, the probiotic-fortified sea buckthorn beverage, designed in our lab, showed efficient pathogen clearance when tested against enteropathogenic *E. coli* and *Salmonella* (Sireswar et al. 2017b).

A plethora of fruit and vegetable matrices have been screened as potential probiotic carrier. In addition to probiotic viability, the recent trend is to also evaluate whether the matrix is able to support the production of useful metabolites, for example, Espirito-Santo et al. (2015) evaluated apple, grape, and orange juice and reported that apple juice not only supported adequate fermentation of *Lb. plantarum* and *Lb. rhamnosus* growth but also showed folate production and SOD activity. Several workers have tried to circumvent the problem of non-supportive matrices, where the probiotic viability was low due to physicochemical properties of the matrix, by blending fruit and vegetable juices. Mauro et al. (2016) reported the development of a blueberry-carrot juice blended beverage containing *Lb. reuteri* as the active culture. Similar blended matrix has been created with pineapple, apple, and mango juices to support the growth of *L. casei* (Mashayekh et al. 2016). Apart from the conventional fruits, several exotic fruits have been explored for non-dairy product development. The Iranian cornelian cherry juice was applied to support *L. casei* T4, though the pH of the juice had to be raised from 2.6 to 3.5 (Nematollahi et al. 2016). Similarly, Andean tubers like oca (*Oxalis tuberosa*), papalisa (*Ullucus tuberosus*), and potato (*Solanum tuberosum* spp. andigena) were fermented with *L. brevis* CJ25 strain (Mosso et al. 2016). A functional cultured beverage was developed using the liquid coconut water containing *Lb. plantarum* by Prado et al. (2015). Later Dharmasena et al. (2015) could increase the shelf life up to 7 weeks by supplementing the coconut water with oatmeal.

Another trend has been to use the vegetable tissue for probiotic and mineral fortification. Very recently, Genevois et al. (2017) demonstrated that pumpkin tissues can be used for fortification of iron and *L. casei*. Though the probiotic survived for 14 days, the customer acceptability of such a product is yet to be evaluated. Similarly, cut and whole fruit and vegetables like apples and olives have also been reported. An interesting study was reported by Jabłońska-ryś et al. (2016) on *Lb. plantarum* fermented button mushroom (*Agaricus bisporus*) fruiting body. Similarly, several minimally processed cut fruits have been probiocated, for example, fresh-cut cantaloupe was inoculated with riboflavin producing *Lb. plantarum* B2 and *L. fermentum* PBCC 11.5. The melon pieces showed a shelf stability of 11 days (Russo et al. 2015). Low-cost pulses like mung bean have been extracted into mung bean milk and have been applied as probiotic matrix for *Lb. plantarum* (Wu et al. 2015). Since a majority of the fruit and vegetable matrices pose the problem of low viability of the probiotic strain in the products, microencapsulation and spray drying techniques are being evaluated as a possible solution. However, the commercial feasibility of applying these techniques has to be rigorously evaluated.

Cereal Matrices

The origin of cereal or whole grain-based probiotic products can be traced to Japan and Europe. Several fermented cereal products exist which have the potential to be transformed into probiotic beverages, for instance, boza, kvass, pozol, ben-saalga, degue, kenkey, koko, kanun-zaki, mageu, mawe, munkoyo, bushera, ogi, thobwa, ting, and uji. However, the discussion in this chapter will be restricted to commercial probiotic products which are sold in different parts of the world.

Encouraging results have been obtained from evaluations of oats, maize, barley, wheat, and malt as matrices that support the growth of human lactobacillus strains (Angelov et al. 2006; Laine et al. 2003). As alternative matrix for probiotic foods, cereals have the advantage of large distribution and nutritive value. Cereals contribute to significant percentage (73%) of global harvested area and of global food production (FAO 2006). Apart from macronutrients, cereals also contain phenolic compounds, phytosterol, essential fatty acids, and resistant starch. The presence of these functional compounds in cereals has been related to lower risks of CVD and other chronic diseases, thereby popularizing whole cereal consumption. However, ability to support probiotic growth and to sustain their viability during storage conditions greatly differs from matrix to matrix. For instance, a comparative evaluation of barley, wheat, and barley malt extracts showed that malt extract performed as the best matrix that supported the growth and viability of human derived *Lb. plantarum* strain during refrigerated storage (Charalampopoulos and Pandiella 2010). The reasons cited for better performance by malt extract were the presence of protective compounds like raffinose and dietary fibers in the matrix. The potential of oat matrix for development of probiotic products has been evaluated by several research groups (Kedia et al. 2008; Angelov et al. 2006).

While in the past extensive research was done on screening of cereal matrices that would support probiotic growth and viability, more recently scientists have also started investigating the physicochemical reactions of probiotication and how it affects the final product. It is also important to remember that market success of a new probiotic formulate is not only dependent on health-promoting properties but also its organoleptic attributes. It is often noticed that the sensory attributes of a new product strongly influence the consumer behavior toward the product. Sensorial profile includes color, consistency, flavor and aroma, texture, mouthfeel, aftertaste, etc. In case of fermented cereals-probiotic products, it is expected that the flavor and aroma profile will undergo major changes after product development and during storage. Salmeron and coworkers reported the aroma profile of four cereal matrices, viz., oat, wheat, barley, and malt fermented with *Lb. plantarum*, *Lb. reuteri*, *and Lb. acidophilus* (Salmeron et al. 2014, 2015). The predominant volatile compounds detected were oleic acid, linoleic acid, acetic acid, and 5-hydroxymethylfurfural in oat, wheat, barley, and malt, respectively. The stages at which flavor compounds may have been generated are during milling of cereals, Maillard reaction, sterilization, enzymatic hydrolysis, and fermentation process. These studies confirmed that different cereal matrices contribute to its own unique flavor profile and that the microorganisms did not significantly contribute to the profile. Over the years, a systematic approach for process optimization has been opted by authors. Gupta et al. (2010) applied the Box-Behnken optimization tool for development of oat-based product fermented with *Lb. plantarum*; the process variables like percentage oat, sugar, and inoculum were optimized using the design.

Therefore, high-fiber cereal substrates not only add nutritional value to the resulting product; they also support the complex nutritional requirements of probiotic strain. It also appears that suitable manipulations of the processing steps can further enhance the sensorial qualities of the product.

Meat Matrices

Originally, in the meat matrix, probiotics were incorporated as protective cultures as a part of hurdle technology for food safety. Over the years, meat matrix has evolved as an adaptable probiotic carrier especially since it was shown that probiotic stains added in meat matrix could render the probiotic bile tolerant. Klingberg and Budde (2006) confirmed that the sausage matrix forms a protective environment for probiotic strain during the GIT transit. Like other food matrices, meat also contains bioactive compounds like conjugated linoleic acid, carnosine, anserine, L-carnitine, glutathione, taurine, and creatine (Arihara 2006). Dry fermented meat products are usually not heated or only mildly heated making it a suitable probiotic carrier.

The greatest challenge for the probiotication or probiotic fortification is that the probiotic strain should be able to survive and dominate the other fermentative organisms present in the product. The probiotic culture to be used should be salt and nitrite resistance since these ingredients are often used for curing of meat. The strain chosen should have limited or no biogenic amines production and have the ability to suppress amine-producing microflora. One must also address the lipid and protein oxidation resulting in color loss during value addition of meat product with probiotics. The quality of the final product (e.g., ham, loin, sausage) is closely related to ripening and storage. The process favors the growth of probiotic microorganisms. Past reports show that Iberian dry fermented sausage (Ruiz-Moyano et al. 2008) and Scandinavian-type fermented sausage (Klingberg et al. 2005) are good candidates for probiotic delivery vehicles.

The probiotication of meat is not yet common since industry has to overcome technological limitations. Nevertheless, recent studies have been reported on evaluation of probiotic fortification of different types of meat matrices (Rouhi et al. 2013; Sidira et al. 2015; Swtwiwathana and Visessanguan 2015; Neffe- Skocinska et al. 2015). It appears that probiotic meat is poised to become an important segment in meat processing industry once the existing scientific and technological gaps are bridged.

Market Trends

The global probiotic market is still in the development stage and is expected to exhibit increasing growth as people become more health conscious and are switching to preventative healthcare mainly due to rising healthcare costs. The increase in population and disposable incomes in developing Asian countries is driving the demand for functional foods and dietary supplements, which in turn serves as a driver for the probiotic ingredients market. Extensive investments by major industries in R&D for novel product portfolio have been in progress for several years now. Usually the companies outsource the product development to third party which has expertise in development of probiotic strain for market entry. Leading companies such as Yakult Honsha Co., Ltd. (Japan) and Chr. Hansen A/S (Denmark) have developed patented strains of microorganisms (probiotics) claiming to have specific health benefits.

The probiotic ingredients market is segmented on the basis of application into food and beverage, dietary supplements, and animal feed. The food and beverage segment has been further classified into dairy, bakery and confectionery, dry foods, non-dairy beverages, meat, and cereals.

Probiotic food and beverage segment was the largest segment in 2015 and accounted for more than 85% of total revenue. This application includes dairy products, non-dairy products, cereals, baked food, fermented meat products, and dry food probiotics. Dairy products accounted for a major share in 2015, and this trend is expected to continue from 2016 to 2024. Human probiotics accounted for more than 90% of total revenue in 2015 (Grandview Research 2016) Fermented meat products segment is expected to show the highest growth rate over the forecast period.

The probiotic products market was valued at $33.19 Billion in 2015. It is projected to reach $46.55 Billion by 2020 growing at a CAGR of 7.0%. In 2014, the market was dominated by Asia-Pacific, followed by Europe. The Asia-Pacific market is projected to grow at the highest CAGR, with rapid growth in the food and beverage industry in developing countries such as Japan, India, and China. The Middle East and Africa and Central and South America are also expected to show modest growth over the forecast period (Markets and Markets 2016). Increasing clinical evidence pertaining to efficiency in disease treatment and maintaining health will drive probiotic market growth in the overall healthcare sector. Curing intestinal inflammation, urogenital infections, and antibiotic against diarrhea by fighting bad gut bacteria are among the key properties facilitating product penetration.

Need for standardized labeling parameters and efficient packaging owing to its perishable nature are among the common challenges for the industry participants. Probiotic market price trend is highly dependent on its raw material and R&D cost.

The industry expects significant growth in this food segment owing to treatments associated with lipid metabolism, lactose intolerance, oxalate metabolism, inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), ulcerative colitis, eczema, allergic rhinitis, *Helicobacter pylori*, necrotizing enterocolitis, and infectious diarrhea.

Global Products

A market survey of the globally available products reveals that non-dairy probiotic products are slowly finding its way into our lives and into our daily diets (Tables 9.1, 9.2, and 9.3). The heterogeneous food matrices of fruit and vegetables and cereals are no longer constraints for novel product development. The consumers are now being offered a shelf diversity and refreshing change from the otherwise dairy-dominated product milieu; it is especially coming from the fruit and vegetable sector. Due to technological advances, several shelf-stable non-dairy products have been created not only from cereals and fruit and vegetables but also non-conventional matrices like chewing gum, sugar, water, tea, kombucha tea, and baking mix. It is also interesting to note that along with *Lactobacillus*, another genus which is being used more extensively in commercial products is *Bacillus coagulans* and its spores because of its thermophilic nature.

Global Regulatory Environment

The global regulatory environment regarding probiotics is not uniform. It varies from region to region. Japan, by the virtue of FoSHU (food for specified health use), has had the longest established market for probiotics; it also permits health claims for a large number of different strains. Asia-Pacific is by far the most permissive legislation. Australia and New Zealand also have permissive regulatory environment where health claims are made on case-to-case basis. Toward the west, the USA and Canada also have fairly lenient regulatory requirements. The Canadian Govt. has a list of approved “probiotics” and allows general health claims like “provides microorganisms that contribute to a healthy gut flora (Degnan 2008). In Latin American Chile, allows labels like ‘may help stimulate the immune system.” The regulations at EU are stricter. There are no approved health claims for probiotic organisms except for “aiding lactose digestion.” Major industry participants include Danisco A/S, Danone, Chr. Hansen, Nestle, Arla Foods, Inc., Probi, and Lallemand Inc. (Arora and Baldi 2015).

The future success of probiotic products would depend upon consumer awareness, faith on their efficacy, and safety.

Table 9.1 Global commercial probiotic products based on fruit and vegetable matrices

Serial No.	Food matrix	Commercial name	Origin	Active probiotic culture
1	Fruit juice	Garden of Flavor Probiotic Juice	USA	*B. coagulans*
2	Fruit soda	Obi, Probiotic Soda	USA	*B. coagulans*
3	Fruit juice	Biola	Norway	*Lb. rhamnosus*
4	Fruit juice	Valio Bioprofit	Finland	*Lb. rhamnosus*
5	Fruit juice	Rela by Biogaia	Sweden	*Lb. reuteri*
6	Cold pressed fruits and vegetables	Welo Probiotic cold pressed drink	Canada	*B. coagulans*
7	Fruit drink	Probi-Bravo Friscus	Sweden	*Lb. plantarum and Lb. paracasei*
8	Fruit drink	Valio Gefilus	Finland	*Lb. rhamnosus* GG
9	Fruits and vegetables	Pressery's organic probiotic soup	USA	*B. coagulans*
10	Fruit drink	Danone-ProViva	Sweden, Finland	*Lb. plantarum*
11	Fermented organic sugarcane molasses infused with specialized herbal tea	Vita Biosa 10+	Canada	*B. animalis*, *B. lactis*, *B. longum*, *Lb. acidophilus*, *Lb. casei*, *Lb. rhamnosus*, *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subs. lactis biovar. diacetylactis*, *L. pseudomesenteroides*, *S. thermophilus*
12	Fruit juice	Tropicana Essentials Probiotics	USA	*B. lactis*
13	Dried plums	Mariani Premium Probiotic Prunes	California, USA	*B. coagulans*
14	Fruits and vegetable smoothie	Love Grace Probiotic Smoothie	New York City, USA	*B. coagulans*
15	Pressed water with organic fruits, vegetables	Suja Pressed Probiotic Waters	California, USA	*B. coagulans*
16	Raw organic fruits and vegetable blend	Garden of Life RAW Organic Kids Probiotic	Florida, USA	*Lb. gasseri*, *Lb. plantarum*, *B. lactis*, *Lb. casei*, *Lb. acidophilus*
17	Fruit juice drink	GoodBelly	Colorado, USA	*Lb. plantarum* 299v

(continued)

Table 9.1 (continued)

Serial No.	Food matrix	Commercial name	Origin	Active probiotic culture
18	Fruit juice	Bravo Easy Kit for Fruit Juice	Mendrisio, Switzerland	*Lb. salivarius*, *Lb. acidophilus*, *Lb. casei*, *Lb. rhamnosus*, *Lactococcus lactis*, *Bifidobacterium*
19	Fruit and vegetable based	KeVita active probiotic drink	Oxnard, USA	*B. coagulans*, *Lb. rhamnosus*, *Lb. plantarum*
20	Fruit juice	Healthy Life Probiotic juice	Australia	*Lb. plantarum*, *Lb. casei*
21	Mango juice	Naked, 100% mango juice with probiotics	California, USA	*Bifidobacterium*
22	Fruit-infused water	Uncle Matt's cold pressed water	Clermont, Florida	*B. coagulans*
23	Fruit juice	Harvest soul, probiotic juice	Atlanta	*B. coagulans*
24	Fruit juice	Oasis, health break probiotic juice	Toronto, Ontario	*Lb. rhamnosus*
25	Passion fruit	Body Ecology Passion Fruit Biotic	USA	*Lb. acidophilus and Lb. delbrueckii*
26	Turmeric and ginger root	Welo probiotic ferments	Canada	*B. coagulans*
27	Dried plums	Mariani Premium Probiotic Prunes	California, USA	*B. coagulans*

Table 9.2 Global commercial probiotic products based on cereal matrices

Serial No.	Food matrix	Commercial name	Origin	Active probiotic culture
1	Burritos	Sweet Earth Natural foods-**Get Cultured!™** Probiotic Burritos	California, USA	*B. coagulans*
2	Poppers	Brad's Broccoli Poppers	Pipersville, PA	*B. coagulans*
3	Cereal bar	Welo Probiotic Bar	Canada	*B. coagulans*
4	Oatmeal bar	Pop Culture Probiotic	California, USA	*B. coagulans*
5	Muesli	Nutrus Slim Muesli	India	*B. coagulans*
6	Muesli	Something to Crow About Probiotic Muesli	New Zealand	*B. coagulans*
7	Cereal bar	Macrolife Macrogreens Superfood Bars	California, USA	*Lb. acidophilus*, *Lb. rhamnosus*, *Lb. bulgaricus*, *B. longus*, *B. breve*

(continued)

Table 9.2 (continued)

Serial No.	Food matrix	Commercial name	Origin	Active probiotic culture
8	Probiotic + prebiotic bar	Truth Bars	USA	*B. coagulans*
9	Cereal bar	Vega One, All-in-One Meal Bars	USA	*B. coagulans*
10	Cereal bar	EffiFoods Probiotic CareBars	USA	*B. coagulans*
11	Cereal bar	Good! Greens Bars	USA	*B. coagulans*
12	Cereal bar	PROBAR Meal Bars	Salt lake City, UT	*B. coagulans*
13	Baking mix	Enjoy Life Foods	Chicago, USA	*B. coagulans*
14	Probiotic granola bar	Udi's Gluten Free	USA	*B. coagulans*
15	Vegan protein with probiotics	Swanson GreenFoods Formulas	Fargo, North Dakota	Not found
16	Cereals	Yog Active Probiotic Cereals	Germany	*Lb. acidophilus*

Table 9.3 Global commercial probiotic products based on other matrices

Serial No.	Food matrix	Commercial name	Origin	Active probiotic culture
1	Chewing gum	ProDenta	Stockholm	*Lb. reuteri* Prodentis
2	Sugar	+Probiotic sugar 2.0	California, USA	*B. coagulans*
3	Cultured coconut milk yogurt	SO DELICIOUS®	North America and Europe	*Lb. bulgaricus*, *Lb. plantarum*, *Lb. rhamnosus*, *Lb. paracasei*, *B. lactus and S. thermophilus*
4	Coconut cream and pea protein	Daiya	Canada	*Lb. plantarum*, *Lb. casei*
5	Almond milk	Kite Hill, almond milk yogurt	California, USA	*S. thermophilus*, *Lb. bulgaricus*, *Lb. acidophilus* and *Bifidobacterium*
6	Organic grains and wild crafted coconuts	CocoBiotic	Australia	*Lb. acidophilus* *Lb. delbrueckii*
7	Berry flavored gummies	Rainbow Light Probiolicious Gummies	USA	*Lb. sporogenes*
8	Kombucha	KeVita Master Brew Kombucha	Oxnard, USA	*B. coagulans*
9	Baking mix	Enjoy Life Foods	Chicago, USA	*B. coagulans*
10	Tea	Bigelow Lemon Ginger Herb Plus Probiotic Tea	USA	*B. coagulans*

Concluding Comments

Given the tight profit margins in the broader food industry and the fact that dairy sector has limited options to offer, the only road ahead for the manufacturers is to look toward exploring the non-dairy food matrices for creating new food brands. The reason for quicker development in dairy sector was the lack of challenge; the matrix was inherently supportive of probiotic cultures. But now, as we look back, it is being realized that dairy matrices do not have the advantage of sustainable or prolonged novelty which may be offered by matrices like fruits and vegetables or meat. Much has been said about technological complexities and challenges of non-dairy food formats, but despite them, several commercially viable products are already star attractions of multi-brand retail shops. Thus it would be safe to say that non-dairy probiotic products that have arrived are here to stay for a long time. Some food formats like meat will require several more years of rigorous research; nevertheless, these products will also rise to the level of a commercially successful product in near future.

As a result of continued advertising and combining marketing schemes, significant consumer awareness has been generated for several probiotic products. Time has come when manufacturers should use this to increase market placing and visibility of non-dairy products.

The key to success will be to convince consumers to pay the cost for a new product, and it can be achieved only through communication of unambiguous and truthful health claims to the consumers.

References

Angelov A, Gotcheva V, Kuncheva R et al (2006) Development of a new oat-based probiotic drink. Int J Food Microbiol 112:75–80

Arihara K (2006) Strategies for designing novel functional meat products. Meat Sci 74:219–229

Arora M, Baldi A (2015) Regulatory categories of probiotics across globe. A review representing existing and recommended categorization. Ind J Med Microbiol 33(5):2–10

Charalampopoulos D, Pandiella SS (2010) Survival of human derived *Lactobacillus plantarum* in fermented cereal extracts during refrigerated storage. LWT Food Sci Technol 43(3):431–435

Degnan FH (2008) The US food administration and probiotics: regulatory categorization. Clin Infect Dis 46:S133–S136

Dharmasena M, Barron F, Fraser A et al (2015) Refrigerated shelf life of a coconut water-oatmeal mix and the viability of *Lactobacillus plantarum* Lp 115-400B. Foods 4(3):328–337. https://doi.org/10.3390/foods4030328

Di Cagno R, Coda R, De Angelis M et al (2013) Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiol 33:1–10

Duda-Chodak A, Tarko T, Satora P et al (2015) Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. Eur J Nutr 54(3):325–341

Espirito-Santo AP, Catherine FC, Renard MGC (2015) Apple, grape or orange juice: which one offers the best substrate for lactobacilli growth? – a screening study on bacteria viability, superoxide dismutase activity, folates production and hedonic characteristics. Food Res Int 78:352–360

FAO – Food, Agriculture Organization of the United Nations (2006) Statistical yearbook 2005–2006. FAO, Publishing Management Service, Rome

Genevois C, de Escalada PM, Flores S (2017) Novel strategies for fortifying vegetable matrices with iron and *Lactobacillus casei* simultaneously. LWT Food Sci Technol. https://doi.org/10.1016/j.lwt.2017.01.019

Granato D, Branco GF, Nazzaro F et al (2010) Functional foods and nondairy probiotic food development: trends, concepts, and products. Comp Rev Food Sc Tech 9(3):292–302

Grand View Research (2016) Grand view research. Retrieved December 20, 2016, from Grand View Research. Web site: (http://www.grandviewresearch.com/industry-analysis/probiotics-market)

Gupta S, Cox S, Abu-Ghannam N (2010) Process optimization for the development of a functional beverage based on lactic acid fermentation of oats. Biochem Eng J 52(2–3.) 15):199–204

Jabłońska-ryś E, Sławińska A, Radzki W (2016) Evaluation of the potential use of probiotic strain *Lactobacillus plantarum* 299v in lactic fermentation of button mushroom fruiting bodies. Acta Sci Pol Technol Aliment 15(4):399–407

Kedia G, Vázquez JA, Pandiella SS (2008) Fermentability of whole oat flour, PeriTec flour and bran by *Lactobacillus plantarum*. J Food Eng 89:246–249

Klingberg TD, Budde BB (2006) The survival and persistence in the human gastrointestinal tract of five potential probiotic lactobacilli consumed as freeze-dried cultures or as probiotic sausage. Int J Food Microbiol 109(1–2):157–159

Klingberg TD, Axelsson L, Naterstad K et al (2005) Identification of potential probiotic starter cultures for Scandinavian-type fermented sausages. Int J Food Microbiol 105:419–431

Laine R, Salminen S, Benno Y et al (2003) Performance of *Bifidobacteria* in oat-based media. Int J Food Microbiol 83:105–109

Lee H, Yoon H, Ji Y et al (2011) Functional properties of *Lactobacillus* strains from kimchi. Int J Food Microbiol 145:155–161

Markets and Markets (2016) Research insight: new revenue pockets – probiotics market. Retrieved December 27, 2016, from Markets and Markets. http://www.marketsandmarkets.com/ResearchInsight/probiotic-ingredient.asp.

Mashayekh S, Hashemiravan M, Mokhtari FD (2016) Study on chemical and sensory changes of probiotic fermented beverage based on mixture of pineapple, apple and mango juices. J Curr Res Sci 4(3):1–5

Matilla-Sandholm T, Myllarinen P, Crittenden R, Mogensen G, Fonden R, Saarela M (2002) Technological challenges for future probiotic foods. Int Dairy J 12:173–182

Mauro CSI, Guergoletto KB, Garcia S (2016) Development of blueberry and carrot juice blend fermented by *Lactobacillus reuteri* LR92. Beverages 2(4):37–40. https://doi.org/10.3390/beverages2040037

Mosso AL, Lobo MO, Cristina Sammán N (2016) Development of a potentially probiotic food through fermentation of Andean tubers. LWT Food Sci Technol 71:184–189

Neffe-Skocińska K, Jaworska D, Kołożyn-Krajewska D et al (2015) The effect of LAB as probiotic starter culture and green tea extract addition on dry fermented pork loins quality. Bio Med Res Int, Article ID 452757. doi.org/10.1155/2015/452757

Negi B, Dey G (2009) Comparative analysis of total phenolic content in sea buckthorn wine and other selected fruit wines. World Acad Sci, Eng Technol Int J Biol Biomol Agric Food Biotechnol Eng 3(6):300–303

Nematollahi A, Sohrabvandi S, Mohammad A et al (2016) Viability of probiotic bacteria and some chemical and sensory characteristics in cornelian cherry juice during cold storage. Electron J Biotechnol 21:49–53

Ozdal T, Sela DA, Xiao J et al (2016) The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. Forum Nutr 8(2):78–83

Prado FC, Lindner JDD, Inaba J et al (2015) Development and evaluation of a fermented coconut water beverage with potential health benefits. J Funct Foods 12:489–497

Puupponen-Pimiä R, Aura AM, Oksman-Caldentey KM et al (2002) Development of functional ingredients for gut health. Trends Food Sci Technol 13(1):3–11

Rahavi EB, Kapsak WR (2010) Health and wellness product development. In Prepared foods network http://www.prepared foods.com/Articles/Feature_Article/BNP_GU ID_9-5--2006_A_10000000000000752310. Access on 18 Sept 2010

Rouhi M, Sohrabvandi S, Mortazavian AM (2013) Probiotic fermented sausage: viability of probiotic microorganisms and sensory characteristics. Crit Rev Food Sci Nutr 53(4):331–348. https://doi.org/10.1080/10408398.2010.531407

Ruiz-Moyano S, Martín A, Benito MJ et al (2008) Screening of lactic acid bacteria and bifidobacteria for potential probiotic use in Iberian dry fermented sausages. Meat Sci 80:715–721

Russo P, Peña N, de Chiara MLV et al (2015) Probiotic lactic acid bacteria for the production of multifunctional fresh-cut cantaloupe. Food Res Int 77:762–772

Salmerón I, Thomas K, Pandiella SS (2014) Effect of substrate composition and inoculum on the fermentation kinetics and flavour compound profiles of potentially non-dairy probiotic formulations. LWT Food Sci Technol 55(1):240–247

Salmerón I, Thomas K, Severino S et al (2015) Effect of potentially probiotic lactic acid bacteria on the physicochemical composition and acceptance of fermented cereal beverages. J Funct Foods 15:106–115

Selma MV, Espín JC, Tomás-Barberán FA (2009) Interaction between phenolics and gut microbiota: role in human health. J Agric Food Chem 57(15):6485–6501

Sidira M, Kandylis P, Kanellaki M et al (2015) Effect of immobilized *Lactobacillus casei* on volatile compounds of heat treated probiotic dry-fermented sausage. Food Chem 178(1):201–207

Sireswar S, Dey G, Dey K et al (2017a) Evaluation of probiotic *L. rhamnosus* GG as a protective culture in sea buckthorn-based beverage. Beverages 3(4):48–54

Sireswar S, Dey G, Sreesoundarya TK et al (2017b) Design of probiotic-fortified food matrices influence their antipathogenic potential. Food Biosci 20:28–35

Swetwiwathana A, Visessanguan W (2015) Potential of bacteriocin-producing lactic acid bacteria for safety improvements of traditional Thai fermented meat and human health. Meat Sci 109:101–105

Valdés L, Cuervo A, Salazar N et al (2015) The relationship between phenolic compounds from diet and microbiota: impact on human health. Food Funct 6:2424–2439

Vitali B, Minervini G, Rizzello CG et al (2012) Novel probiotic candidates for humans isolated from raw fruits and vegetables. Food Microbiol 31:116–125

Wu H, Rui X, Li W et al (2015) Mung bean (Vigna radiata) as probiotic food through fermentation with *Lactobacillus plantarum* B1-6. LWT Food Sci Technol 63(1):445–451

Chapter 10
Technological Interventions in Fermented Meat Production: The Commercial Perspective

Nevijo Zdolec

Abstract Selected microbiological and chemical-toxicological hazards associated with the production of fermented meat products are discussed in terms of technological interventions required for their reduction and control. The complexity of this production imposes the need for a strict implementation of good hygiene and manufacturing practices, given that a number of hazards occur and transfer along the food chain, while some are generated by technological interventions undertaken during meat processing. Hazards of the first kind arise due to the microbiota resistant to antimicrobial agents, while hazards of the second kind arise due to the presence of biogenic amines, mycotoxins and polycyclic aromatic hydrocarbons. Nevertheless, public health importance of fermented meat products is neither lesser nor greater than that of other foodstuffs. Challenging technological measures and risk control strategies give an added value to this kind of meat products.

Keywords Fermented meat products · Technological interventions · Public health · Antimicrobial resistance · Mycotoxins · PAH · Biogenic amines

Introduction

Fermented meat products are the most popular group of meat products produced using both traditional techniques and industrial technologies. Historically, their production has gradually developed in different parts of the world, eventually making these products indigenous (autochthonous), that is to say, typical of different geographical regions. In the ancient times, maturation of fermented meat products took place under atmospheric conditions (climate, seasonal temperatures, air flows,

N. Zdolec (✉)
University of Zagreb, Faculty of Veterinary Medicine, Department of Hygiene, Technology and Food Safety, Zagreb, Croatia
e-mail: nzdolec@vef.hr

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_10

winds, smoking, relative humidity) crucial for the development of specific organoleptic properties (Oiki et al. 2017). Nowadays, this production has been standardized and transferred into better controlled environments.

The production of fermented sausages and cured meat is quite a challenging process conditioned by numerous external and internal factors that can significantly affect the quality and safety of the final product. The above factors include product ingredients (meat, fatty tissue and additives), salting/curing, casing, sausage batter preparation, brine, microclimate and the composition and activity of autochthonous microflora or starter cultures added into the product. As the commercially most valuable meat products, fermented sausages are produced from the highest-quality meat, while cured meat products are produced from certain parts of the animal's body (leg, loin, neck, etc.).

Fermented meat products can be analysed and discussed from various standpoints: microbiological, technological, chemical, biochemical, toxicological, nutritional and so forth, however, always in view of specific public health issues. The complexity of the production and health aspects associated with fermented meat products requires a multidisciplinary approach that gives a broad insight into potential risks and their control (Zdolec 2017). For the purposes of this chapter, certain microbiological and chemical-toxicological hazards associated with the production of fermented meat products have been selected to be discussed, together with technological interventions that may affect them. When addressing microbiological risks associated with this type of meat products, this chapter shall mainly focus on antimicrobial resistance issues. The latter issues shall not be discussed in relation to food-borne pathogens or transmission along the food chain but rather in the context of naturally present non-pathogenic microbiota that may also be carriers of mobile resistance determinants. In this respect, the recently adopted GRAS (generally recognized as safe) status of lactic acid bacteria is being reviewed (Vesković-Moračanin et al. 2017). Since fermented meat products fall into the ready-to-eat (RTE) food category, the importance of good hygiene practices, i.e. the prevention of contamination of raw materials and ingredients, including contamination with potentially resistant bacteria, is evident. An additional technological intervention capable of reducing or eliminating resistant bacteria in/from fermented meat products (sausages) would be the use of competitive starter cultures able to colonize the meat substrate and therefore prevent the growth of potentially harmful microbiota. The latter particularly applies to enterococci as a part of the LAB population naturally found in fermented meat (Čop 2016). Enterococci are not necessarily the indicators of faecal contamination, since they are ubiquitous, i.e. present everywhere in the environment. For example, multiresistant strains have been isolated from raw milk collected from the udders of healthy cows that have never been treated with antibiotics but did cohabitate with treated animals (Zdolec et al. 2016). This may indicate the presence of a resistant enterococci population in the biosystem, which is very important in view of hygienic procedures exercised within the frame of food production, especially the production of RTE foodstuffs.

Further microbiological hazards associated with fermented meat production include environmental contaminants or contaminants generated during meat processing, such as mycotoxins, biogenic amines or polycyclic aromatic hydrocarbons. In general, mycotoxins are considered to be contaminants longitudinally transmitted along the food chain, that is to say, from feed to food. As regards the fermented meat production, direct contamination with toxigenic moulds can be expected under poor hygienic conditions and from environmental sources (Pleadin and Bogdanović 2017). A poor hygienic practice also results in the accumulation of biogenic amines in fermented meat products. The strategy of selection and application of starter cultures that inhibit the development of aminogenic microbiota seems appropriate in controlling the risk of biogenic amines' presence in fermented meat products (Lorenzo et al. 2017).

Finally, the presence of harmful PAHs in/on smoked fermented meat products is conditioned by smoking technologies and factors such as wood combustion temperature and oxygen content (high temperature and oxygen absence = high levels of harmful PAH compounds), wood species and its humidity (soft and moist wood = more PAH compounds) and the duration of smoking (Šimko 2005).

This chapter shall discuss technological interventions that can upgrade the safety of fermented meat products and shall refer to selected potential hazards – antimicrobial resistance of indigenous bacteria, biogenic amines, mycotoxins and polycyclic aromatic hydrocarbons.

Fermentation as a Part of Hurdle Technologies: A Sausage Example

Fermentation is one of the oldest methods of meat preservation, a process dependent on metabolic activity of fermentative microorganisms. By virtue of fermentation and acidification, changes in meat proteins occur, which also affect the water loss rate and sausage drying. It should be mentioned that fermentation that takes place under controlled conditions (in ripening chambers) is carried out at higher temperatures for a shorter period of time as compared to traditional production. Accordingly, different and complex microbiological, biochemical and physicochemical changes occur and progress, affecting the quality and safety of the final products (Gandemer 2002). The level of initial microbial contamination depends on microbiological quality of ingredients and (non-)hygienic approach exercised during the production. However, due to the basic physicochemical processes taking place in certain ripening stages (lowering pH and water activity and increasing the salt content), the sausage mixture profile contributes to the development of specific microbiota (acidophilic, halophilic, osmophilic). It has been long known that the most active microorganisms in fermented sausages are LAB (e.g. lactobacilli) and staphylococci/micrococci. In addition, certain types of fermented sausages are characterized by the presence of a stable population of yeasts, moulds and enterococci. All of them affect, separately and in association with tissue enzymes, the development of desirable sausage sensory properties (taste, smell, colour, aroma, consistency). The principle of metabolic activity of the abovementioned microorganisms has been established and can be identified in each meat substrate, but the diversity of fermented sausages' production techniques, raw materials and ingredients and microclimate or macroclimate conditions greatly affects the affirmation of certain microbial species or strains (Zdolec et al. 2008).

Autochthonous Microbiota and Starter Cultures

The quality of traditional fermented sausages derives from spontaneous fermentation taking place due to the activity of indigenous microorganisms. The above imposes the need for the monitoring of microbial ecology of traditional sausages and the selection of strains having optimal technological and safety properties (Danilović and Savić 2017). Spontaneous fermentation can also result in potentially hazardous products, in terms of presence of antibiotic-resistant bacteria, biogenic amines, enterotoxins and pathogenic bacteria (Zdolec 2017). By selecting and applying competitive starter cultures, technological and safety advances can be achieved in terms of product uniformity, quality standardization and reduction of the existing microbiological risks.

Industrial-scale production of fermented sausages largely depends on the selection of appropriate starter cultures. Despite many approved starters present on the market, the selection of new functional LAB strains is still intensive and focused on natural sources of potential starter cultures (Holck et al. 2017). Traditionally fermented sausages fermented by autochthonous "wild" microbiota are rich in LAB having favourable technological and hygienic properties suitable for food-related applications. Their phenotypic and genotypic characterization should include a wide range of technological, health and safety assessment criteria (Zdolec 2012). Some studies suggest that the application of autochthonous cultures may result in the upgraded quality of commercial products produced using standard industrial starters (Frece et al. 2014). In addition, dominant autochthonous LAB strains isolated from a specific fermented meat product should be considered as potential functional starter cultures for the same product. However, domination of some LAB species/strains (i.e. hetero-fermentative LAB) could result in products of poorer quality and/or lesser safety. For this reason, the selection of potential starters should involve all potential risk assessment criteria, such as the assessment of toxigenicity, acquired transmissible antimicrobial resistance or technologically unacceptable pathways (Zdolec et al. 2013a).

Microbiological Hazards and Technological Interventions

Fermented meat products are generally stable and safe food products as regards the presence of pathogenic microbiota, but their stability and safety still depend on the type of product, its physicochemical properties and any subsequent contamination that might have occurred. In general, different technologies and their combinations known as the "hurdle concept" can be used for microbiological risks' control (Kamenik 2017). The most numerous and biologically most active groups of microorganisms present in certain types of fermented meat products are LAB, staphylococci, micrococci, yeasts and moulds, all of them potentially suitable for use as starter cultures. The purpose of adding a starter culture is to uniform the product quality (sensory properties) and accelerate the production process. One of the possibilities of reducing undesirable microbiota in fermented products is the use of protective cultures that exhibit antimicrobial activity or the use of their antimicrobial metabolites such as bacteriocins (Fraqueza et al. 2017). In recent years, studies have highlighted the importance of antimicrobial resistance of natural microbiota present in fermented meat products and proposed strategies (including competitive starters) to combat this public health problem along the food chain (Fraqueza 2015; Zdolec 2017).

Combating the Resistance of Autochthonous Microbiota Present in Fermented Meat Products

The resistance of bacteria to antimicrobial substances (antibiotics and chemotherapeutics) is one of the most important problems in veterinary public health and food safety. The resistance of pathogenic bacteria present in food of animal origin is systematically monitored and viewed upon as a current public health problem longitudinally transmitted along the food chain, from animals to humans. In addition to traditional zoonotic bacteria, the role of commensal non-pathogenic bacteria in antimicrobial resistance transfer should be taken into account. In this respect, it is also important to monitor the development of antimicrobial resistance in non-pathogenic bacteria inhabiting animal and human intestines and non-pathogenic bacteria present in food of animal origin.

In addition to pathogenic bacteria, antimicrobial resistance is an issue associated with non-pathogenic bacteria, too; namely, the latter may transfer resistance genes along the food chain. Although the presence of LAB and coagulase-negative staphylococci (CNS) represents the "conditio sine qua non" for fermented meat production, some of these strains may possess hazardous properties, including antimicrobial resistance determinants. Table 10.1 shows the results of some studies on LAB resistance in fermented meat products performed worldwide.

Multiresistant enterococci are frequently found in fermented meat products and other fermented foods. Opinions on their hygienic and technological acceptability

Table 10.1 Selected studies on LAB antimicrobial resistance in fermented meat products (modified from Zdolec et al. 2017a)

Food, country	LAB	Resistance	Reference
Fermented sausages, Spain	*Lb. sakei* *Lb. plantarum* *Lb. paracasei* *Lb. coryniformis* *E. faecium*	Vancomycin, rifamycin, amikacin, tetracycline	Landeta et al. (2013)
Fermented sausages, China	*Lb. plantarum* *Lb. fermentum* *Lb. helveticus* *E. faecium*	Tetracycline, erythromycin, chloramphenicol, kanamycin	Pan et al. (2011)
Fermented sausages, Italy	*Lb. sakei* *Lb. curvatus* *Lb. plantarum*	Tetracycline, erythromycin	Zonenschain et al. (2009)
Dry sausages, cured ham, Canada	*E. faecalis* *E. faecium* *E. gallinarum*	Clindamycin, tetracycline, tylosin, erythromycin	Jahan et al. (2013)
Fermented sausages, Portugal	*E. faecalis* *E. faecium* *E. casseliflavus*	Rifampicin, tetracycline, erythromycin, ciprofloxacin	Barbosa et al. (2009)
Fermented sausages, ham, Germany	*E. faecalis* *E. faecium*	Enrofloxacin, erythromycin, avilamycin, quinupristin/dalfopristin (*E. faecium*), tetracycline, erythromycin (*E. faecalis*)	Peters et al. (2003)
Fermented sausages, Turkey	*E. faecalis* *E. faecium*	Erythromycin, tetracycline, kanamycin	Toğay et al. (2010)

are usually dual; on one hand, these bacteria improve sensory properties of a product, but, on the other hand, they are also spoilage bacteria, opportunistic pathogens and carriers of resistance genes. Enterococci can survive and multiply in meat products during fermentation, especially in products in which competitive starter cultures were not added (traditional fermented meat products) (Zdolec et al. 2017b). Some studies showed a continuous increase in enterococci population during the course of sausage fermentation, while other studies failed to detect any enterococci during the same course (Danilović and Savić 2017). Recently, a dairy-originated probiotic culture of *Enterococcus faecalis* was inoculated into the sausage batter (10^5 CFU/g), showing an acceptable adaptability to the meat substrate (Zdolec et al. 2017b). However, Cocconcelli et al. (2003) reported a high risk of acquired antimicrobial resistance in food-inhabiting enterococci and a high risk of transfer of mobile genetic material to other bacteria, even with low antibiotic pressure. Some studies showed the exchange of genetic material responsible for antimicrobial resistance, which goes on between clinical and food isolates and emerges during meat fermentation (Jahan et al. 2015; Gazzola et al. 2012).

Coagulase-negative staphylococci are also naturally present in various types of food, including fermented meat products, in which they contribute to the development of sensory properties by virtue of their lipolytic and proteolytic activity. However, recent research has shown the presence of opportunistic pathogenic species during spontaneous (natural) fermentation of meat, such as *Staphylococcus epidermidis* (Marty et al. 2012), which often carries resistance genes (Martin et al. 2006; Resch et al. 2008; Zdolec et al. 2013b).

As reported above, classical microbiological hazards (food-borne pathogens) are less often expected to be present in fermented meat products due to the known hurdles used in fermented meat technology. Lactic acid bacteria are even a part of the hurdle concept but, controversially, have also been recognized as a part of the antimicrobial resistance problem. Nevertheless, their significance in human nutrition (probiotics) and food technology (starter cultures) should not be questioned. However, there is no barrier to prevent a development of acquired resistance between pathogens (e.g. streptococci), opportunistic pathogens (e.g. enterococci) and commensal LAB (e.g. intestinal lactobacilli, lactococci), as apparent from the presence of identical resistance determinants in all microbial groups (Mathur and Singh 2005). Strategies aiming at the reduction of antimicrobial resistance in fermented meats' microbiota should be based on the prudent use of antimicrobials in food animals and the application of competitive starter cultures during meat fermentation (Zdolec et al. 2017a).

Mycotoxins in Fermented Meat Products and Technological Interventions

Mycotoxins are toxic substances produced by certain types of moulds. It is known that, under favourable conditions (temperature, humidity, oxygen), moulds can grow almost everywhere, even on animal feed as well as on the surface of meat products during their drying and ripening. In general, moulds have a very important technological role in the production of fermented meat products and significantly contribute to specific sensory properties of the latter products. They can be inoculated onto a sausage surface by virtue of immersing the product into a solution or by virtue of spraying; the moulds used for the purpose are mainly those of the *Penicillium* species (Tabanelli et al. 2012). Such "desirable" moulds are white or white-grey, while undesirable moulds are black, green or yellowish (Feiner 2006). The mould layer applied on the sausage surface contributes to uniform drying, slows the moisture loss down and protects the product from discoloration and rancidity (Incze 2010). However, most often in natural indigenous production, risks stemming from the development of toxigenic moulds under the existing hygienic and environmental conditions may be witnessed (Oiki et al. 2017). Certain additional factors, such as casing damage, may also affect an undesirable mould growth (Pleadin et al. 2015a). Markov et al. (2013) reported that dry-fermented meat products produced by individual households were most frequently contaminated with *Penicillium* moulds, while the most commonly detected mycotoxin was ochratoxin A. Pleadin et al. (2015b) claimed that the highest levels of ochratoxin A found in sausages and cured meat products under their study were five to ten times higher than the recommended 1 μg/kg.

Pleadin and Bogdanović (2017) reported the main conditions favouring mycotoxin contamination of the final products, as follows: (i) nonstandardized production quality and technology, (ii) environmental mould (spore) contamination as a consequence of automated ripening chambers' non-use, (iii) the use of raw meat contaminated through animal feedstuffs and contaminated spices and contamination arising as a consequence of activity of toxin-producing moulds overgrowing the product surface and (iv) inability to remove mycotoxins using standard production and preservation technologies. Furthermore, technological operations such as thermal processing, curing, drying and ripening, as well as storage conditions, do not have a significant impact on the reduction of mycotoxin levels in the final product (Pleadin et al. 2014; Pleadin and Bogdanović 2017).

Biogenic Amines Present in Fermented Meat Products and Technological Interventions

Biogenic amines (BAs) are biologically active compounds produced via decarboxylation of free amino acids or via amination and transamination of aldehydes and ketones (Maijala et al. 1993). BAs can be detected in all types of fermented foods, including fermented meat products. The production of biogenic amines in foodstuffs depends on the presence of free amino acids and the degree of bacterial decarboxylase activity. The most common biogenic amines associated with food poisoning are cadaverine, putrescine, histamine, spermidine, spermin, tyramine and tryptamine (Marijan et al. 2014; Sahu et al. 2015).

The accumulation of biogenic amines in fermented meat products is facilitated by the use of contaminated raw materials or processing under poor hygienic conditions. Tyramine, cadaverine, putrescine and histamine are biogenic amines most frequently found in fermented meat products (Ruiz-Capillas and Jiménez-Colmenero 2004; Pleadin and Bogdanović 2017). For instance, the tyramine and the putrescine content found in different European fermented sausages ranged from 76 to 187 mg/kg and from 33 to 125 mg/kg, respectively (Ansorena et al. 2002). Besides microbiological factors, the amine content in fermented sausages also depends on pH, temperature, salt, sausage type (size and diameter) and starter cultures' activity (Latorre-Moratalla et al. 2008). Large-diameter sausages usually contain higher amounts of BAs. The selection of proper starter cultures to be used in the production of fermented sausages has a crucial role in reducing biogenic amines. Lorenzo et al. (2017) stated that measures to reduce the formation of BAs during fermented meat products' production should focus on the control of aminogenic microbial groups. The measures proposed in this regard can be divided into three groups: (i) quality control of raw materials in order to minimize the microbial load of meat and other ingredients and additives, (ii) the use of appropriate starter cultures to the effect of spoilage microbiota control and (iii) the use of spices and/or additives and the control over environmental conditions during fermentation/ripening.

Chemical-Toxicological Hazard: Polycyclic Aromatic Hydrocarbons (PAH) Present in Fermented Meat Products

Smoking is a part of both traditional and industrial production technology of certain fermented meat products (Lücke 2017). In traditional ways, smoke is generated by a slow combustion wood burning, subsequently applied to hanging meat products in the form of a hot smoke, or by a firebox separated from the smoking chamber. It is well known that natural smoke contributes to the development of favourable organoleptic properties of sausages and cured meats, improving their aroma, flavour, colour and taste; such a smoke possesses antimicrobial and antioxidant properties, as well (Feiner 2006). However, in food safety concepts, smoke is considered to be a potential source of harmful compounds, such as polycyclic aromatic hydrocarbons (PAH), which may be found on the surface of or inside smoked foods (Šimko 2002; Ozcan et al. 2011).

The content of benzo[a]pyrene (BaP) in food is a good indicator of the total PAH amount. BaP concentration currently allowed in food is 2 μg/kg, as compared to the former limit of 5 μg/kg (Directive EC 1881/2001; Regulation EC 835/2011). However, a more appropriate indicator of PAH presence risk in smoked meat and meat products is the sum of benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene, representing the PAH4, allowed to be present in the concentration of 12 μg/kg. Besides PAH4, the datum useful for the PAH presence-related risk assessment is the sum of 8 PAHs, i.e. the sum of the PAH4, benzo[k]fluoranthene, benzo[g,h,i]perylene, dibenzo[a,h]anthracene and indeno[1,2,3-c,d]pyrene (EFSA 2008).

The content of PAHs in smoked meat products depends on several factors, such as smoking technology, casing permeability, fat content, smoking wood type, the presence of oxygen, moisture content and wood-burning temperatures (Šimko 2005; Stumpe-Vīksna et al. 2008; Gomes et al. 2013). Studies of the benzo[a]pyrene content in traditionally or industrially smoked meat products revealed the compliance with the food safety criteria (<2 μg/kg) and the fact that the most prevalent PAHs are harmless low molecular weight PAHs like naphthalene, acenaphthene, fluorine, phenanthrene or anthracene (Djinovic et al. 2008; Roseiro et al. 2011;

Santos et al. 2011; Lorenzo et al. 2011; Gomes et al. 2013; Škrbić et al. 2014). However, higher PAH concentrations have been found in traditionally smoked meat products as compared to those obtained using controlled industrial smoking technologies (Roseiro et al. 2011; Škrbić et al. 2014).

Public health significance of PAHs is manifested through their mutagenic and carcinogenic properties, particularly strong in compounds composed of five or more rings, while low molecular weight PAHs are harmless (Šimko 2005). PAHs are stable and persistent in the environment and animal fat tissues and transferable through the food chain (Dobríková and Světlíková 2007). Pažin et al. (2016) detected significant amounts of PAHs in a sausage mixture before smoking (116.43 μg/kg). Roseiro et al. (2011) found nearly the same PAH content in a raw material used in the production of traditional Portuguese fermented sausages (106.17 μg/kg). In contrast, Djinovic et al. (2008) reported a significantly lower PAH content in industrially produced sausages before smoking (2 and 3.5 μg/kg). The study of Pažin et al. (2016) showed that the content of individual and total PAHs continuously increases during the smoking period, followed by the decrease in the same content towards the end of drying. Šimko et al. (1991) reported that the increase in BaP and total PAH contents could be expected due to the sausage drying and moisture loss; however, this increase is followed by a decrease occurring because of photodegradation. The same authors emphasized that the BaP content does not change significantly in dry matter and that the PAH content in smoked meat products strongly depends on environmental conditions, like oxygen and light availability. Several studies showed that the BaP content in traditionally smoked dry sausages exceeds tolerant values, which is not the case in products produced under controlled industrial conditions (Šimko 2002). Wretling et al. (2010) found up to 36.9 μg/kg BaP in “sauna-smoked” meat, i.e. meat directly exposed to hot smoke. However, when a separate smoke generator was used, the BaP content was acceptable. Smoking of household-produced sausages using cold smoke distributed from a separate firebox has been shown as an appropriate method as regards the BaP quantity and the total PAH content determined in such products (Pažin et al. 2016).

Some alternative processes have been implemented in order to reduce the PAH content in smoked meat products; the latter include the filtration of particles, the use of cooling traps, smoking at lower temperatures and/or process shortening. The extended use strategy is characterized by the incorporation of smoke flavourings produced from primary products obtained from different woods under specific pyrolytic conditions and their extraction into meat products in the range of 0.1–1.0% (Toldrá and Reig 2007; Pleadin and Bogdanović 2017).

Conclusion

Fermented meat products are well-accepted by consumers due to their high nutritional value, attractive organoleptic properties and today’s accelerated lifestyle. At the same time, consumers are aware of known health-related disadvantages of these products, such as high levels of salt, fat or cholesterol. These products are microbiologically stable, but potential hazards can arise from the presence of natural microbiota resistant to antibiotics or contaminants arising during meat processing, such as mycotoxins or biogenic amines. In addition, it is also necessary to monitor toxicological-chemical indicators of product safety, such as polycyclic aromatic hydrocarbons. However, fermented meat products are burdened by potential public health hazards and risks neither less nor more than other types of foods. Challenging technological measures and risk control strategies give an added value to this kind of meat products.

References

Ansorena D, Montel MC, Rokka M, Talon R, Eerola S, Rizzo A, Raemaekers M, Demeyer D (2002) Analysis of biogenic amines in northern and southern European sausages and role of flora in amine production. Meat Sci 61:141–147

Barbosa J, Ferreira V, Teixeira P (2009) Antibiotic susceptibility of enterococci isolated from traditional fermented meat products. Food Microbiol 26:527–532

Cocconcelli PS, Cattivelli D, Gazzola S (2003) Gene transfer of vancomycin and tetracycline resistance among *Enterococcus faecalis* during cheese and sausage fermentation. Int J Food Microbiol 88:315–323

Čop M (2016) Influence of *Enterococcus faecalis* 101 home-made dry sausage quality. Graduate thesis, Faculty of veterinary medicine, University of Zagreb

Danilović B, Savić D (2017) Microbial ecology of fermented sausages and dry-cured meat. In: Zdolec N (ed) Fermented meat products: health aspects. CRC Press, Boca Raton, pp 127–166

Djinovic J, Popovic A, Jira W (2008) Polycyclic aromatic hydrocarbons (PAHs) in different types of smoked meat products from Serbia. Meat Sci 80:449–456

Dobríková E, Světlíková A (2007) Occurrence of benzo[a]pyrene in some foods of animal origin in the Slovak Republic. J Food Nutr Res 46:181–185

EFSA (2008) Polycyclic hydrocarbons in food. Scientific opinion of the panel on contaminants in the food chain. The EFSA J 724:1–114

Feiner G (2006) Meat products handbook. Practical science and technology. CRC Press, Boca Raton

Fraqueza MJ (2015) Antibiotic resistance of lactic acid bacteria isolated from dry-fermented sausages. Int J Food Microbiol 212(6):76–88

Fraqueza MJ, Patarata L, Laukova A (2017) Protective cultures and bacteriocins in fermented meats. In: Zdolec N (ed) Fermented meat products: health aspects. CRC Press, Boca Raton, pp 228–269

Frece J, Kovačević D, Kazazić S, Mrvčić J, Vahčić N, Delaš F, Ježek D, Hruškar M, Babić I, Markov K (2014) Comparison of sensory properties, shelf life and microbiological safety of industrial sausages produced with autochthonous and commercial starter cultures. Food Tech Biotech 52:307–316

Gandemer G (2002) Lipids in muscles and adipose tissues, changes during processing and sensory properties of meat products. Meat Sci 62:309–321

Gazzola S, Fontana C, Bassi D, Cocconcelli PS (2012) Assessment of tetracycline and erythromycin resistance transfer during sausage fermentation by culture-dependent and –independent methods. Food Microbiol 30:348–354

Gomes A, Santos C, Almeida J, Elias M, Roseiro LC (2013) Effect of fat content, casing type and smoking procedures on PAHs contents of Portuguese traditional dry fermented sausages. Food Chem Toxicol 58:369–374

Holck A, Axelsson L, McLeod A, Rode TM, Heir E (2017) Health and safety consideration of fermented sausages. J Food Qual 2017.: Article ID 9753894:25
Incze K (2010) Mold-ripened sausages. In: Toldra F (ed) Handbook of meat processing. Blackwell Publishing Professional, Ames, pp 363–378
Jahan M, Krause DO, Holley RA (2013) Antimicrobial resistance of enterococcus species from meat and fermented meat products isolated by a PCR-based rapid screening method. Int J Food Microbiol 163:89–95
Jahan M, Shanel GG, Sparling R, Holley RA (2015) Horizontal transfer of resistance from *Enterococcus faecium* of fermented meat origin to clinical isolates of *E. faecium* and *Enterococcus faecalis*. Int J Food Microbiol 199:78–85
Kamenik J (2017) Hurdle technologies in fermented meat production. In: Zdolec N (ed) Fermented meat products: health aspects. CRC Press, Boca Raton, pp 95–126
Landeta G, Curiel JA, Carrascosa AV, Muñoz R, DeLas RB (2013) Technological and safety properties of lactic acid bacteria isolated from Spanish dry-cured sausages. Meat Sci 95:272–280
Latorre-Moratalla ML, Veciana-Nogués T, Bover-Cid S, Garriga M, Aymerich T, Zanardi E, Ianieri A, Fraqueza MJ, Patarata L, Drosinos EH, Lauková A, Talon R, Vidal-Carou MC (2008) Biogenic amines in traditional fermented sausages produced in selected European countries. Food Chem 107:912–921
Lorenzo JM, Purriños L, Bermudes R, Cobas N, Figueiredo M, García Fontán MC (2011) Polycyclic aromatic hydrocarbons (PAHs) in two Spanish traditional smoked sausage varieties: "chorizo gallego" and "chorizo de cebolla". Meat Sci 89:105–109
Lorenzo JM, Franco D, Carballo J (2017) Biogenic amines in fermented meat products. In: Zdolec N (ed) Fermented meat products: health aspects. CRC Press, Boca Raton, pp 450–473
Lücke F-K (2017) Fermented meat products - an overview. In: Zdolec N (ed) Fermented meat products: health aspects, CRC Press, Boca Raton, pp 1–14.
Maijala RL, Eerola SH, Aho MA, Hirn JA (1993) The effects of GDL-induced pH decrease on the formation of biogenic amines in meat. J Food Prot 56:125–129
Marijan A, Džaja P, Bogdanović T, Škoko I, Cvetnić Ž, Dobranić V, Zdolec N, Šatrović E, Severin K (2014) Influence of ripening time on the amount of certain biogenic amines in rind and core of cow milk Livno cheese. Mljekarstvo 64(3):59–69
Markov K, Pleadin J, Bevardi M, Vahčić N, Sokolić-Mihalek D, Frece J (2013) Natural occurrence of aflatoxin B1, ochratoxin A and citrinin in Croatian fermented meat products. Food Control 34:312–317
Martin B, Garriga M, Hugas M, Bover-Cid S, Veciana-Nogués MT, Aymerich T (2006) Molecular, technological and safety characterization of gram-positive catalase-positive cocci from slightly fermented sausages. Int J Food Microbiol 107:148–158
Marty E, Buchs J, Eugster-Meier E, Lacroix C, Meile L (2012) Identification of staphylococci and dominant lactic acid bacteria in spontaneously fermented Swiss meat products using PCR-RFLP. Food Microbiol 29:157–166
Mathur S, Singh R (2005) Antibiotic resistance in food lactic acid bacteria – a review. Int J Food Microbiol 105(3):281–295
Oiki H, Kimura H, Zdolec N (2017) Traditional production of fermented meats and related risk. In: Zdolec N (ed) Fermented meat products: health aspects. CRC Press, Boca Raton, pp 49–57
Ozcan T, Akpinar-Bayizit A, Irmak Sahin O, Yilmaz-Ersan L (2011) The formation of polycyclic hydrocarbons during smoking process of cheese. Mljekarstvo 61:193–198
Pan L, Hu X, Wang X (2011) Assessment of antibiotic resistance of lactic acid bacteria in Chinese fermented foods. Food Control 22:1316–1321
Pažin V, Šimunić Mežnarić V, Tompić T, Hajduk G, Legen S, Zdolec N (2016) Polycyclic aromatic hydrocarbons in smoked fermented sausages from household. Proceedings 6th Croatian veterinary congress, Opatija, Croatia, pp 579–588
Peters J, Mac K, Wichmann-Schauer H, Klein G, Ellerbroek L (2003) Species distribution and antibiotic resistance patterns of enterococci isolated from food of animal origin in Germany. Int J Food Microbiol 88:311–314

Pleadin J, Bogdanović T (2017) Chemical hazards in fermented meat. In: Zdolec N (ed) Fermented meat products: health aspects. CRC Press, Boca Raton, pp 417–449
Pleadin J, Perši N, Kovačević D, Vulić A, Frece J, Markov K (2014) Ochratoxin A reduction in meat sausages using processing methods practiced in households. Food Addit Contam Part B 7:239–246
Pleadin J, Kovačević D, Perši N (2015a) Ochratoxin A contamination of the autochthonous dry-cured meat product "Slavonski Kulen" during a six-month production process. Food Control 57:377–384
Pleadin J, Malenica Staver M, Vahčić N, Kovačević D, Milone S, Saftić L, Scortichini G (2015b) Survey of aflatoxin B_1 and ochratoxin A occurrence in traditional meat products coming from Croatian households and markets. Food Control 52:71–77
Resch M, Nagel V, Hertel C (2008) Antibiotic resistance of coagulase-negative staphylococci associated with food and used in starter cultures. Int J Food Microbiol 127:99–104
Roseiro LC, Gomes A, Santos C (2011) Influence of processing in the prevalence of polycyclic aromatic hydrocarbons in a Portuguese traditional meat product. Food Chem Toxicol 49:1340–1345
Ruiz-Capillas C, Jiménez-Colmenero F (2004) Biogenic amines in meat and meat products. Crit Rev Food Sci 44:489–499
Sahu L, Panda SK, Paramithiotis S, Zdolec N, Ray RC (2015) Biogenic amines in fermented foods: overview. In: Montet D, Ray RC (eds) Fermented foods part I: biochemistry and biotechnology. CRC Press, Boca Raton, pp 318–332
Santos C, Gomes A, Roseiro LC (2011) Polycyclic aromatic hydrocarbons incidence in Portuguese traditional smoked meat products. Food Chem Toxicol 49:2343–2347
Šimko P (2002) Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. J Chromatogr B 770:3–18
Šimko P (2005) Factors affecting elimination of polycyclic aromatic hydrocarbons from smoked meat foods and liquid smoke flavorings. Mol Nutr Food Res 49:637–647
Šimko P, Karovičová J, Kubincová M (1991) Changes in benzo [a]pyrene content in fermented salami. Z Lebensm Unters Forsch 192:538–540
Škrbić B, Đurišić-Mladenović N, Mačvanin N, Tjapkin A, Škaljac Š (2014) Polycyclic aromatic hydrocarbons in smoked dry fermented sausages with protected designation of origin *Petrovska klobasa* from Serbia. Maced J Chem Chem En 33:227–236
Stumpe-Vīksna I, Bartkevičs V, Kukāre A, Morozovs A (2008) Polycyclic aromatic hydrocarbons in meat smoked with different types of wood. Food Chem 110:794–797
Tabanelli G, Coloretti F, Chiavari C, Grazia L, Lanciotti R, Gardini F (2012) Effects of starter cultures and fermentation climate on the properties of two types of typical Italian dry fermented sausages produced under industrial conditions. Food Control 26:416–426
Toğay SO, Keskin AC, Açik L, Temiz A (2010) Virulence genes, antibiotic resistance and plasmid profiles of enterococcus faecalis and enterococcus faecium from naturally fermented Turkish foods. J Appl Microbiol 109:1084–1092
Toldra F, Reig M (2007) Chemical origin toxic compounds. In: Toldra F (ed) Handbook of fermented meat and poultry. Blackwell Publishing, Ames, pp 469–475
Vesković-Moračanin S, Djukić D, Zdolec N, Milijašević M, Mašković P (2017) Antimicrobial resistance of lactic acid bacteria in fermented food. J Hyg Eng Des 18:25–35
Wretling S, Eriksson A, Eskhult GA, Larsson B (2010) Polycyclic aromatic hydrocarbons (PAHs) in Swedish smoked meat and fish. J Food Comp Anal 23:264–272
Zdolec N (2012) Lactobacilli – functional starter cultures for meat fermentation. In: Peres Campos AI, Mena AL (eds) Lactobacillus: classification, uses and health implications. Nova Sci Pub, New York, pp 273–289
Zdolec N (2017) Fermented meat products: health aspects. CRC Press, Boca Raton
Zdolec N, Hadžiosmanović M, Kozačinski L, Cvrtila Ž, Filipović I, Škrivanko M, Leskovar K (2008) Microbial and physicochemical succession in fermented sausages produced with bacte-

riocinogenic culture of *Lactobacillus sakei* and semi-purified bacteriocin mesenterocin Y. Meat Sci 80(2):480–487
Zdolec N, Dobranić V, Horvatić A, Vučinić S (2013a) Selection and application of autochthonous functional starter cultures in traditional Croatian fermented sausages. Int Food Res J 20(1):1–6
Zdolec N, Račić I, Vujnović A, Zdelar-Tuk M, Matanović K, Filipović I, Dobranić V, Cvetnić Ž, Špičić S (2013b) Antimicrobial resistance of coagulase negative staphylococci isolated from spontaneously fermented sausages. Food Tech Biotech 51(2):240–246
Zdolec N, Dobranić V, Butković I, Koturić A, Filipović I, Medvid V (2016) Antimicrobial susceptibility of milk bacteria from healthy and drug-treated cow udder. Vet Arhiv 86(2):163–172
Zdolec N, Vesković-Moračanin S, Filipović I, Dobranić V (2017a) Antimicrobial resistance of lactic acid bacteria in fermented meat products. In: Zdolec N (ed) Fermented meat products: health aspects. CRC Press, Boca Raton, pp 319–342
Zdolec N, Čop M, Dobranić V (2017b) Implementation of dairy-origin culture of *Enterococcus faecalis* 101 in the production of dry sausages. Hrvatski veterinarski vjesnik 25(1–2):56–62
Zonenschain D, Rebecchi A, Morelli L (2009) Erythromycin- and tetracycline-resistant lactobacilli in Italian fermented dry sausages. J Appl Microbiol 107:1559–1568

Chapter 11
Modernization of Fermenters for Large-Scale Production in the Food and Beverage Industry

Steve Carly Zangué Desobgo

Abstract Fermentation is an inexpensive and low-energy method of conserving decomposable unprocessed materials. Many foods have microbial sources or include components generated by fermentation using microorganisms. This procedure is helpful for extending the usability of food and drinks, as well as improving the health benefits of products. This chapter examines the modern aspects of food and beverage fermentation in large-scale fermenters. Descriptions of fermenters and their underlying technology are provided for each case to demonstrate the link between the equipment and the process. Engineering characteristics are reported for bioreactor scale-up, including important problems in the scaling and exploitation of bioreactors. Agitation in bioreactors is also discussed. Some aspects associated with bioreactor/fermenter design when computer simulation is used are reported. Finally, the technology of modern bioreactor types is presented.

Keywords Fermentation · Microorganism · Scaling up · Bioreactor · Food · Beverage · Technology

Introduction

Fermenting is a biotechnological unit operation involving microbes. Through fermenting, raw sustainable substrates are changed to added-value products, such as fermented beverages and food, enzymes, alcohols, acids, and others. The production of commercial fermented products has improved from focused genetic

S. C. Z. Desobgo (✉)
Department of Food Process and Quality Control, University Institute of Technology of the University of Ngaoundere, Ngaoundere, Cameroon

Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, Johannesburg, South Africa
e-mail: desobgo.zangue@gmail.com

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_11

engineering methods that created modern microbial strains (Campbell-Platt 1994). The development of end or subsidiary products is reliant on the selected microbial variety and ecological conditions, such as bioreactor type. For an ideal fermenting procedure, the microbe variety should be chosen and created in light of the desired end product. Information on the biochemical modifications of matured foods can help producers to control their results by modifying varieties and conditions. In addition to development conditions, media, strains, and fermenting methods influence outcomes and hence profitability. Fed-batch, continuous, and batch fermentation systems can be selected for high efficiency. Continuous and fed-batch systems can handle substrate restriction amid fermentation procedures. Higher efficiency also can be achieved by the immobilization of cells, which can be derived incrementally from the biomass collection in the bioreactor, leading to an expanded assembly of biocatalysts in the bioreactor.

Modern large-scale fermentation procedures can vary throughout the beverage and food industry. The focal point of these procedures are generally bioreactors, which can be categorized by the bioreactor feeding operation (continuous, fed-batch, or batch), fixation of the biocatalyst, stirring system of the bioreactor (hydraulic, pneumatic, or mechanical agitation), and the accessibility of oxygen (anaerobic, microaerobic, or aerobic), among others. The choice of bioreactor or fermentation technique for a specific application should consider the advantages and inconveniences of every setup. This decision should include the properties and accessibility of essential crude materials, any important venture and working costs, manageability, accessibility of a skilled workforce, and the desired efficiency and quantifiable profit (Inui et al. 2010). In large-scale applications, every fermentation technique needs to run effectively and dependably; thus, a significant aspect in the choice of a fermentation/bioreactor procedure is the capital expenses per unit of item recuperated. However, even with a plan and effective operation, issues with the subsidiary product and wastewater administration are unavoidable in large-scale operations.

This chapter is focuses on the aspects of fermenters in food manufacturing, biotechnological approaches, and the modernization and design of bioreactors in the industry.

Modern Fermenters for Food and Beverages

Beer Fermenter Technology

Fermenter for Lager Beers

Worldwide, most beer is manufactured using a variety of lager *Saccharomyces carlsbergensis* yeast, which has a tendency drift away from the base of the fermentation vessel. Thus, the tanks used for the fermentation of lager beer—which are the most well-known and critical in brewing—should take into consideration this yeast property.

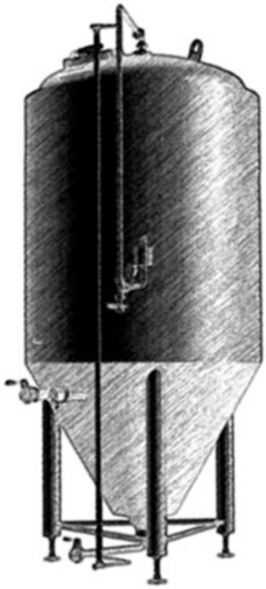


Fig. 11.1 Fermentation and lagering tank

A noteworthy achievement in fermenter configuration was the implementation of closed fermenters. Leopold Nathan patented his plans for cylindrical tanks, which were vertical with funnel-shaped bases, in 1908 and 1927. Modern versions of the Nathan tank are now the most used fermenters. The size of these tanks range from 100 to 6000 hL. An important attribute of these tanks is their well-defined cone at the bottom. An inclination of 70° is necessary for the yeast to subside into the bottom of the tank toward the end of fermentation (Fig. 11.1). This design allows most of the yeast to be isolated, resulting in a relatively yeast-free lager. In a few designs, improvements and storage can occur in one tank from fermentation, without centrifugation of the brew during transfer to another tank for development. A number of advantages have been noted for cylindroconical tanks compared with round or open square fermenters (Briggs et al. 2004), including a reduction in capital expenses of 25–35%, a reduction in operating expenses of 50–65%, reduction of product losses, expanded vessel usage, less introduction of bitter compounds, reduced space requirements, and potential for the collection of CO_2.

Lager fermenters are generally three to four times shorter in their diameter than the height and use an operation pressure of 1–1.5 bar. European fermentation tanks for lager beer commonly use shorter tanks with a diameter-to-height ratio of <2:1, which causes fermentation to equalize more completely than in horizontal vessels. In taller tanks with a proportion of >3:1, there is an tendency for increased generation of more alcohol to the detriment of esters. Expanding the dimensions of the tank can decrease the expenses per unit volume; doubling the dimensions of the tank results in a cost increment of approximately 35% (Briggs et al. 2004).

Fig. 11.2 Stainless steel fermenter tank

Cylindroconical tanks cannot be completely filled for fermentation. A substantial volume of froth is created by the advancement of CO_2, which can cause the pressure discharge valves to clog. Initially, fermentation tanks for lager beer were steel with a glass or epoxy tar lining. However, this coating must be regularly inspected to ensure its integrity. Furthermore, mellow steel is inclined to rust; thus, present-day wtanks are typically made of stainless steel using chrome-nickel (Fig. 11.2). Usually, 304 stainless is used; however, V2A steel is not completely impervious to chloride particles or to pH <4.5. This is not typically an issue for fermenters when solutions with an elevated chloride substance 316 can be determined; however, it is significantly costlier than 304 steels.

The fermenter should be outfitted with a cooling attribute to release the heat created during fermentation and to enable required temperature control. The tanks are filled and exhausted from the bottom, which decreases oxygen entrance. Pipes are fitted to the tanks for the addition of wort, evacuation of yeast, and expulsion of lager. There are also pressure and vacuum alleviation systems. In most of today's tank, the vessels used a pipe-work system. The valves are therefore either associated with each tank or gathered into a "valve routing block" to which each tank is associated; the valves are either manually or remotely controlled. Fermenters also need protection. Outside tanks should be protected against the elements, which may differ significantly depending on geographical location. Indoor vessels also need some protection to minimize the need for system temperature control.

Ale Beer Fermenters

Because lager brews are the most common type of beer worldwide, most technological advancements in fermentation have been associated with cylindroconical tanks. However, in the United Kingdom and Ireland, stouts and ales are

Fig. 11.3 Square tank for wheat beer fermentation

the customary beers. These brews are regularly created with top fermenting yeast varieties, similar to some German and Belgian ales. Conventional ale fermentation uses an open solitary tank to ease yeast expulsion, such as Yorkshire vessels (Anon 2008).

Square tanks (Fig. 11.3) from Yorkshire were initially manufactured with stone and slate, with a size up to 50 hL. However, today's Yorkshire squares are almost always manufactured with stainless steel (304) and have capacities up to 850 hL to accommodate the production needs of a modern brewhouse. Yorkshire squares have a lower section that is isolated from the exposed upper part by a slightly slanting deck. Underneath is a progression of channels known as organ funnels, and perhaps a few sewer vents with spines around the edge. On the highest point of the deck, there is an outlet with an embedded attachment to help remove the yeast.

Vinegar Fermentation Tanks

The word *vinegar* comes from the French *vin aigre* ("harsh wine"). The total ethanol concentration (%v/v) and acetic acid concentration (%w/v)—termed the global concentration (GK)—needs to be consistent throughout acetification. The GK yield is the remaining vinegar, indicated as a level of the GK toward the beginning of acetification. There are various factors affecting acetification, including the oxygen, alcohol, and microbes (Hutkins 2006; Adams 1985; Hills 2014). Wine is normally fermented in tanks, although barrels may be used in some cases, especially for white wine. Generally, wine was historically manufactured in simple, solid, nonmetallic, mineral-matter containers called *lagars*, in which the grapes would be crushed. Lagars are still used occasionally in Portugal by a few winemakers.

Fig. 11.4 Frings acetator technology

In the culture strategies for vinegar, microorganisms form a film at the surface between the air and acidifying environment; this appears to be a straightforward process, yet it may experience varying levels of complexity. The fast process of obtaining vinegar is due to the speed of acetification, which can be increased by expanding the area of dynamic microbial film and enhancing oxygen exchange to the acidifying stock. The acidic microorganisms develop as a film on an inactive material pressed into a false-bottomed tank. The acidifying stock is splashed onto the inert material surface and streams down in opposition to the counter-current flow of air. The inert material in which the microbes are packed is typically composed of a lignocellulosic substance, such as sugarcane bagasse, wood fleece, or vine twigs; however, different substances, such as coke, have also been used. The acetic acid stock begins in a marsh at the tank base and reflows up to the point where the desired level of acidity is attained. A quicker reaction rate indicates that the wash was heated during entry via the bed; thus, on the span of the fermenter, some chilling might be required. The procedure is completed in a semi-continuous system to maintain a high acidity level throughout the process, with a large portion of the biomass held inside the bed.

A commonly used fast vinegar procedure, with a controlled temperature and constrained air circulation, will typically acidify a vinegar stock with a GK value of 10 and an underlying ethanol percentage of 3% in 4–5 days. The quickest acetification rates are accomplished by submerged acidification, in which acetification microbes are suspended in a environment that is oxygenated by air sparging. The most powerful commercial system is the Frings Acetator (Fig. 11.4), which uses a self-preparing aerator to achieve extremely efficient oxygen exchange.

To quickly and efficiently submerge a culture, a semi-continuous operation regularly takes 24–48 h. However, this operation requires much more oversight than what is required for simpler methods. The acetification organisms are very vulnerable to air supply interruptions. To survive suspended in a pH environment of 2.5 and an acidity of 10–14%, the microbes require a consistent provision of energy from air. An air supply interruption of just 1 min in a stock with a GK value of 11.35 can completely and permanently halt acetification; that is, the acetification will not continue when air circulation resumes.

Fermenters in Winemaking

Inox tanks started to gain widespread acceptance in the late 1970s. The advantages of this material include ease of cleaning and the capacity to operate in cooling techniques. Two levels of inox have been used in the manufacturing of fermenters. Grade 304 is most suitable for the fermentation of red wine. The superior grade 316, which contains molybdenum, is stronger and more resistant to erosion; this grade is suitable for both white and red wines. The top of inox tanks may be either open or shut, with a fermentation lock. Variable limit tanks are likewise possible and are helpful, even when the tanks are only halfway filled. These tanks have a drifting metal top, held up by an inflatable plastic tire at the edge. Fiberglass tanks are also occasionally used as a less expensive option to inox. In the early twentieth century, many produced replaced their wooden tanks with inox; however, wood is again gaining interest as a material for tank development at some small- and medium-sized wineries. The most common vessels of winemakers are stainless steel and cylindrical (Fig. 11.5) with an inner cover. The cover is held in situ by an expansive

Fig. 11.5 Wine fermenter tank

vinyl cap, allow the top to be safe at any height in the vessel. Most vats have a level base with a low port on one depleting side. These vats should be put on a stand or depleted using gravity. A few models have three legs welded in situ; these vats also usually have a cone-molded base with an additional deplete port in the middle. Some larger tanks have a built-in stainless-steel coat or belt connected over the center for cooling. A glycol cooling unit may be linked to ports on the coat when chilling is desired. Normally, these vessels are costlier than vessels without a chilling coat. The stainless-steel material is exceptionally chemically inactive and completely safe for beverages. Cleaning is simple with the top evacuated. The cover of a variable limit tank has a port that is suitable for a maturation bolt; thus, these vessels can be used as open or shut fermenters.

The fermentation of red wine uses the whole grape. When manufacturing red wine, two facts should be considered. First, during fermentation, yeast generate CO_2 gas and alcohol. As the CO_2 rises into and out of the wine, a portion gets caught in the peels and causes them to float on the wine surface in a mass named "the cap." The cap rises over the surface of the liquid, which causes the must volume to extend in the vessel. Thus, when charging red wine fermentation tanks, one should select a fermenter that it is somewhat larger than the desired final volume of wine. The skins in the "top" should be washed with the fluid wine a few times each day during fermentation. For large volumes, this can be achieved using a procedure called a "pump-over."

White wines are manufactured using only the grape juice. The maceration and detachment of the juice from the solids occurs before the start of fermentation. Because there are no solids, there is no compelling reason to punch the top. However, white wines are exceptionally touchy to oxygen introduction, so a mechanism to restrict oxygen intake is desirable. Consequently, open-top fermenters are not used. Sealed fermenters are the best choice for making white wines.

Bread Proofers

To guarantee reliable outcomes and maintain production schedules, specific equipment may be used to control the speed and characteristics of fermentation in breadmaking. A proofer of dough (also referred to as a proofing oven or box) is a heating chamber used as a part of baking to promote the yeast fermentation of dough through moderate temperatures and managed humidity. Warmer temperatures increase yeast activity, resulting in increased CO_2 creation and a higher, quicker rise of the dough. Dough is usually allowed to rise in the proofer prior to baking, but it can also be used for the principal rise or mass fermentation. Proofer temperatures can range from 70 °F (21 °C) up to 115 °F (46 °C). Bakers often use large temperature- and humidity-managed proofers.

A dough retarder (Fig. 11.6) is a chiller used to self-manage the yeast fermentation while proofing a mixture. Decreasing the dough temperature creates a steady, slow rise with other maturation characteristics, resulting in more elaborate flavors.

Fig. 11.6 Stainless steel automatic dough retarder proofer

In sourdough bread production, chilling declines the wild yeast activity of the Lactobacilli (Gänzle et al. 1998), which creates flavoring from acetic and lactic acid, for example. Sourdough that is chilled prior to baking may have greater sourness. To prevent the batter from drying, a wind stream is maintained in the mixture retarder. Industrial bakers frequently chill the dough at approximately 50 °F (10 °C).

A banneton is a kind of wicker bin used to give structure to molded chunks of bread during sealing. A banneton wicker bin is also called a brotform or proofing bushel. It is regularly used for dough that is too delicate or soft to keep up its shape while rising. The bread is ordinarily removed from these crates prior to baking. Traditionally, these bushels are made from wicker; however, some new proofing crates have been manufactured using rattan, cane, spruce pulp, terracotta, and polypropylene. A banneton sometimes has a material liner (usually cloth) to keep the mixture from adhering to the sides of the container. However, with use, bannetons usually become non-stick because a small amount of flour collects in them. These wicker containers are used both to give shape to the dough and to draw off humidity from the outside layer. Bannetons are available in elliptical or round shapes.

Dairy Product Fermentation

Lactic acid bacteria play an important role in the creation of fermented milk products. They can ferment lactose and produce lactic acid, which decreases the milk pH (Tamime 2008; Park 2009). As a consequence, the pH attains the isoelectric point of the protein (casein), which is the main protein in milk. At that point, the positive and negative charges are in equilibrium. The casein then coagulates and coagulum appears (Tamime 2008; Park 2009; Clark et al. 2009). This causes both biochemical and physical transmutations in the item. When integrated with lactic acid, starter yeasts may produce organic molecules, such as acetaldehyde, ethanol, acetic acid, diacetyl, and exopolysaccharides, which affect the consistency and flavor profiles of the product (Clark et al. 2009; Hutkins 2006). Thus, variants of cultures and production conditions can cause different characteristics for these products.

Cheesemaking Mechanization

Mechanization and automation have been implemented for the large-scale manufacture of cheese. Huge industrial units stocking a few million kilograms of milk each day. The fat-to-casein ratio for cheese can be achieved via mechanized methods. Cheese can be manufactured in mechanized tanks in which the filling process is controlled using a computer. Culture expansion, cutting, cooking, stirring, renneting, and discharging operations also can be automated (Fig. 11.7). Encased double-jacketed tanks are available with mechanical stirring and cutting instruments. The mixing and cutting tools turn on a pole horizontally or vertically with adjustable speed. Computerized cheesemaking processes and the ability to control pH inline also help to reduce labor costs. Numerous tanks are available with a mechanized gel-quality analyzer to systematize the cutting stage. Whey and curd division can be executed on a depleting conveyer. In a cheddaring tower, air pressure and vacuum

Fig. 11.7 Coagulation vat

pressure can be used for the curd pressing, which is salted and processed in a mellowing/salting transporter. This salted curd can be squeezed and shaped into 18-kg pieces in a piece former, followed by automated packing and transportation to the aging room (Chandan 2014).

Yogurt Fermenters

Large amounts of yogurt can be delivered in a series of individual cupboards. This procedure can be mechanized for continuous production through a tunnel organization. However, the idea of continuous yogurt manufacturing is novel. Pallets with pots of yogurt can be set on a conveyor or smooth rollers and pass through a tunnel composed of two areas. Hot air is circled in the tunnel hatching portion. The pallet rate is determined by the rate of the transport line, which in turn is managed by the speed of lactic acid generation in the drain. Toward the completion of the fermentation time, which is proportionate to pH 4.5, the pallets pass through the cooling segment so that the warm air is supplanted by the introduction of cool air. The yogurt is halfway cooled in this area; the final cooling happens in the chill store. Because the yogurt is moving during the hatching/cooling stages, great care should be taken to prevent harm to the coagulum.

An update to the tunnel organization has been described in the literature (Bylund 1995). The loaded containers of the inoculated milk are put in open-plan cartons and separated from each other. The goal is that the coursing warm/chill air in the brooding and chilling stages can reach each individual container and provide precise temperature control (Fig. 11.8). At the point when the predetermined ideal pH (normally 4.5) is achieved, the time has come to begin cooling. The crates (pallets) are motionless during brooding. They are put in the hatching part of the tunnel to discourage handling.

Through differentiation, the coagulum of mixed yogurt is created in mass. The structure of the gel is destroyed earlier or during the chilling and bundling stages. Nevertheless, preparing the drain base for the production of blended yogurt is as depicted previously. The types of fermentation vats used in factories for the creation of blended yogurt include the following:

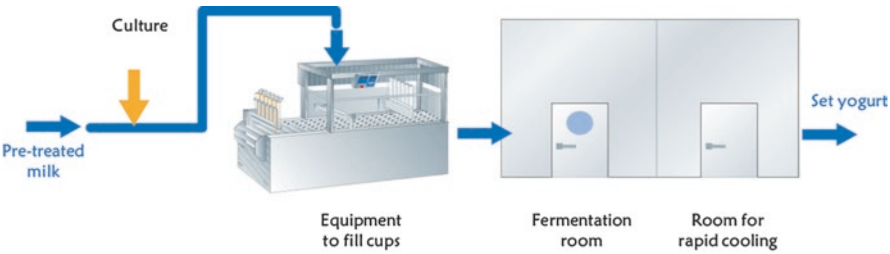


Fig. 11.8 Production of set yogurt

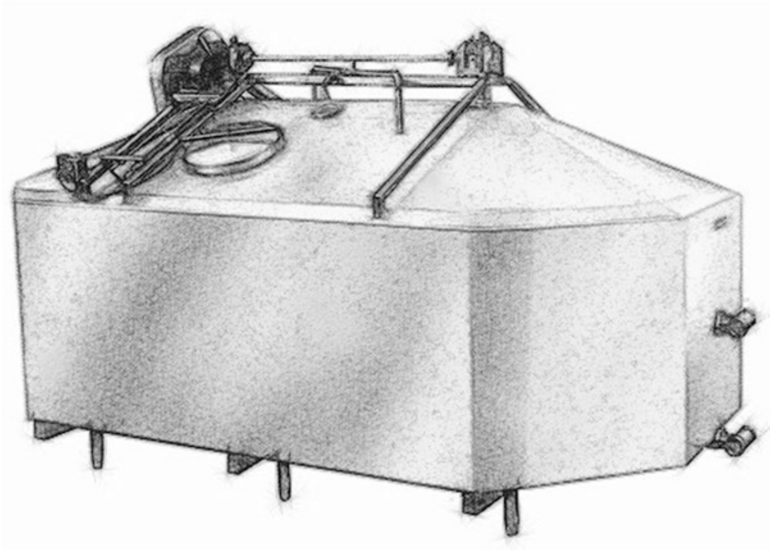


Fig. 11.9 Yogurt coagulation vats

- *Flexible tanks:* A flexible tank is assigned as a number duty vat, with one dedicated to milk fermentation. It is a water-jacketed tank in which vapor can be used during the warming stage; circulating chilled water is used to cool the drain to 40–45 °C. The temperature is kept at 42 °C during the fermentation stage. Finally, chilled water is used to cool the yogurt.
- *Fermentation tanks:* Fermentation tanks are protected, as the end goal is to maintain an even temperature during the hatching stage. The stirring technique in such vats is unforced (Fig. 11.9) because the cone-shaped base encourages simple expulsion of the coagulum (see Fig. 11.9).
- *Chilling/fermentation tanks:* Chilling/fermentation tanks are water jacketed. Hot water (40–45 °C) is circulated during the brooding stage, followed by cold water for incomplete cooling of the coagulum.
- *Sterile fermentation tanks:* Sterile fermentation tanks are modified versions of ordinary fermentation vessels. These vats are utilized for the manufacture of yogurt under germ-free conditions. The vat is protected and suitable for use with a pH electrode and thermometer. The air penetrating and leaving the tank is separated. The essential goal of an aseptic aging tank is to limit contamination of the yogurt with molds and yeast. A sterile tank is always pressurized with germ-free air; a similar approach is used for the generation of mass starters with an aseptic tank.

For protection, each tank is furnished with an additional pipe for air, as well as a security system to keep the vat from exploding from the vacuum created by the drop-in temperature in the wake of cleaning. All of the above vats have a froth-diminishing inlet fitting that mitigates the issue of foam generation in the vat.

Furthermore, most of today's yogurt fermentation vats are fitted with a pH meter to monitor lactic acid generation by the starter (Watanabe et al. 2008; Corrieu et al. 2005; Mulchandani et al. 1995).

Meat Fermenters and Fermentation Technology

The filling of frankfurters takes place in computer-regulated cooling chambers, where they are left to mature for development and improvement of microbes. A typical chamber is shown in Fig. 11.10. Humidity, temperature, and air velocity should be carefully managed to control microbial development and catalyst activity.

Meat maturation technology differs between the United States and Europe. High maturation temperatures (35–40 °C) are common for U.S. frankfurters, followed by a gentle warming procedure as a sort of pasteurization, rather than drying. Hence, cultures such as *Pediococcusacidilactici* or Lactobacillus plantarum, which develop well, are commonly used. In Europe, different technologies might be used, depending on the area and atmosphere. There is a trend to produce short-handled, smoked wieners in chilly and muggy countries (e.g., northern Europe), whereas a prolonged process for dried hotdogs is common in drier and hotter nations (e.g., Mediterranean region).

In northern European countries, frankfurters are aged for around 3 days at transitional temperatures (25–30 °C), followed by a short maturation duration (about

Fig. 11.10 Maturation chamber

3 weeks). These frankfurters are put through a fast drop of pH and are generally smoked for a particular taste (Eva et al. 1997; Demeyer and Stahnke 2002). Mediterranean wieners require a longer duration of preparation. Maturation occurs at moderate temperatures (18–24 °C) for around 4 days, followedd by mellow drying for a more extended time, generally months. *L. curvatus* or *L. sakei* are most frequently used as starters (Toldrá et al. 2001, 2014; Toldrá 2008, 2010). The duration needed for the aging step depends on the temperature and type of microorganisms used.

The process is very different in China and other Asian nations, where frankfurters are first dried at 48 °C for 36 h and afterward at 20 °C for 3 days. Aging is moderately poor; the harsh taste, which is viewed as undesirable, is thus lessened. Chinese crude sausage is eaten in the wake of warming (Leistner 1992).

Vegetable Fermentation Containers

Not all receptacle materials are suitable for maturation. Aging happens in acidic, salty conditions. Metal, aside from high-grade commercial stainless steel, can pit and debase; thus, it is generally not used. Ceramic vessels and plastic receptacles are more stable. Regardless of material, the selected food receptacle should be simple to wash and without profound abrasions, pits, or fragments that can provide a refuge for unsafe microorganisms or influence the aging. Wooden vessels are used to age vegetables; however, they can be challenging to keep intact and sterile. Vessels and other gear should be cleansed in hot, foamy water before use. Chlorine should not be used to clean the equipment because the remaining chloramine and chlorine deposits on the container could affect the development of maturing microbes. If chlorine is used, the equipment should be washed again to eliminate any lingering chemicals.

Ceramic vessels are available in different shapes and sizes. When selecting a container, both the shape and size should be considered. For food safety and adequate maturation, it is vital to have the saline solution/juices at a level of 1–2 in. over the aged vegetables. Such a vegetable plunge allows the items to remain without oxygen, which supports the development of lactic acid microorganisms and other maturing life forms. Keeping the vegetables immersed accounts for the salt to plaster the item, which additionally promotes the development of lactic organisms. Thus, it is critical to select a maturing crock that allows the vegetables to be totally immersed under the saltwater liquid. The style of vessel does not influence the vegetables' security but could influence quality. It is vital to cover the items for both quality and safety.

Plastic vessels also can be used effectively to ferment vegetables, particularly polyethylene vessels with high density (HDPE-2 plastic) that are free of bisphenol and phthalate. These chemicals have been introduced to a few plastics to increase their adaptability; however, they can affect human health, especially for children.

Fig. 11.11 Glass container for anaerobic fermentation of vegetables

Glass vessels (Fig. 11.11) may be used. However, care must be taken to ensure they are not chipped, broken, or split. Quart canning jugs may be used. However, spoilage could be an issue because it can be harder to keep vegetables immersed.

Engineering Aspects for Bioreactor Scale-up

Fundamental Problems in the Design and Operation of Bioreactors

An efficient bioreactor regulates, encloses, and controls a biological reaction. To achieve this, a chemical technician should take account of two items. The first is the appropriate reactor factors for the specified physical, biological, and chemical system. A macro-kinetic (chemical) system embraces microorganism excrescence and matter manufacture. Microorganisms will embrace bacterium, fungi, yeast, and fish, plant, animal, and bug cells, as well as other biological matter. The opposite space of dominant significance in a bioreactor design affects the bioreaction factors, such as restrained temperature, optimum pH, adequate substratum (conventionally a source of carbon; essentially fats, proteins, and sugars), water accessibility, salts as nutrients, vitamins, gas, evolution of gas, and removal of byproducts.

Ideally, a bioreactor should be planned to promote the best physiology of the microorganism, eliminate or reduce pollution from undesirable microbes, and prevent changes to the microorganism. This section provides an outline and summary of bioreaction techniques, the benefits and downsides of a variety of system types, and their everyday applications. A number of subtopics are beyond the scope of this chapter, including biology, sterilization, heat transfer, rheology, mixing, fluidization, surface phenomena, mass transfer and transport enhancements, hydrokinetics, instrumentation, and method management.

Agitation in Bioreactors

The stirring mode within a bioreactor produces the fluid movement that allows many alternative tasks to be achieved. A typical agitated bioreactor is shown in Fig. 11.8. Important features to understand include the interactions between the liquid movement, the agitator momentum, and the power input devoted to the bioreactor and these assignments. In addition, it is necessary to understand how a change to the scale affects these relationships. Several of these aspects are often studied before a bioprocess has been selected; these physical features are most pertinent to microorganism fermentations. The characteristics that are specific to the organism are developed and can sometimes diverge for each case. The features that are more relevant for scale-up are discussed later. The physical aspects have been discussed elsewhere for conditions significant to an extensive variety of life forms (Gupta and Ibrahim 2013; Laskin et al. 2011; Nienow et al. 1997, 2010, 2011; Doran 2013).

This section explores the significance of microbial maturations for which the thickness does not substantially exceed that of water, such as microscopic organisms and yeast. Thus, sticky polysaccharide and filamentous frameworks are excluded from this discussion. With such low thickness, the stream in the bioreactor is turbulent from a 5-L bench bioreactor to the largest scale. That is, the Reynolds number can be calculated as $R_e = \left(\rho_L ND^2 / \mu\right) >\sim 10^4$, where R_e is the development medium thickness (kg/m^3), ρ_L is its consistency (Pa s), D is the impeller length (m), and N is its speed (rev/s). For scale-up, because the stream is turbulent, the substantial estimation of the Reynolds number does not make a difference. Turbulent stream speculations can be used to examine the fluid mechanics in the bioreactors over the scales.

The movement of oxygen in a fermentation broth has been investigated since the 1940s when “submerged fermentations” were first used. The subject was revisited recently (Laskin et al. 2011; Al-Rubeai 2015; Pangarkar 2015). The general oxygen request of the cells throughout the batch or fed-batch fermentation should be met by the oxygen exchange rate and the request increments as long as the quantity of cells is expanding. Generally, for each mole of O_2 used, 1 mol of CO_2 is created (Pangarkar 2015). Subsequently, an extremely high oxygen exchange rate must be achievable. This rate relies upon the mass exchange coefficient, k_La(1/s), and the driving force for mass exchange, ΔC, because

$$\text{OUR} = k_L a \times \Delta C \tag{11.1}$$

Estimations of k_La provide essential data about a bioprocess or bioreactor. These calculations indicate that handling conditions provide a sufficient supply of oxygen for the expansion of cells. Likewise, k_La can be utilized to optimize control factors over the lifecycle of a bioprocess. That optimization would be founded on the oxygen request at different points in time and the development period of the organic matters. Barometers for optimization could include item yield, power utilization, or preparation time.

The oxygen exchange rate of a bioreactor is emphatically affected by the hydrodynamic conditions being utilized as part of the bioprocess (Fig. 11.8). These conditions are a component of energy scattering, which relies on the working conditions, physicochemical characteristics of a culture, geometrical variables of the bioreactor, and the attendance of oxygen-devouring cells.

During cell culture, oxygen is exchanged from a gas (commonly an air pocket or headspace) to a fluid, so it can eventually be ingested into a cell and expended. In the most straightforward terms, this procedure can be depicted as the flux over a hindrance layer communicated as a result of the driving force and mass transport coefficient (k_La) in Eq. 11.2:

$$J^0 = k_G \times \left(p_G - p_i\right) = k_L \times \left(C_i - C_L\right) \tag{11.2}$$

In that condition, J^0 is the oxygen molar flux (mol/m^{2}s) by the means of the gas–liquid network, k_G and k_L are the neighborhood mass-exchange coefficients, p_G is the oxygen halfway weight in the gas stage, and C_L is the broken-down oxygen fixation in the fluid. Index i alludes to values in the gas–liquid network.

Because interfacial focuses are not straightforwardly quantifiable, it is normal to consider a unique instance of Eq. 11.1 that portrays flux under or at harmony:

$$J^0 = k_G \times \left(p_G - p^*\right) = k_L \times \left(C^* - C_L\right) \tag{11.3}$$

In Eq. 11.3, p^* is the oxygen weight in balance with the fluid stage, C^* is the oxygen immersion in the mass fluid, and K_G and k_L are the general mass-exchange coefficients. In any case, when the dissolvability of oxygen in water is low and most mass exchange resistance is on the fluid side of the interface, the general mass-exchange coefficient can be thought to be equivalent to the neighborhood coefficient ($K_L = k_L$). Accordingly, the oxygen mass exchange rate per unit of reactor volume (dCO_2/dt) is acquired by duplicating the general flux of the gas–liquid interfacial range per unit fluid volume using Eq. 11.4:

$$\frac{d\mathrm{CO}_2}{dt} = k_L a \times \left(C^* - C_L\right) \tag{11.4}$$

The mass adjustment for the dispersed oxygen in the very blended fluid stage is portrayed in Eq. 11.5:

$$\frac{dC}{dt} = \text{OTR} - \text{OUR} \tag{11.5}$$

Here, *dC/dt* is the amassing oxygen rate in the fluid stage, *OTR* is the oxygen exchange rate from gas to fluid, and *OUR* is the oxygen take-up rate of the microorganisms. The last term can be calculated by multiplying $qO_2 \times CX$, where qO_2 is the specific oxygen take-up rate of the microorganism utilized and *CX* is the biomass fixation.

Without biomass or with non-respiring cells, biochemical responses do not occur and OUR = 0. For this situation, Eq. 11.5 can be disentangled as follows:

$$\frac{dC}{dt} = k_L a \times \left(C^* - C_L\right) \tag{11.6}$$

Because it is difficult to measure k_L and a independently, the item $k_L a$ is treated as a solitary quantifiable variable: the volumetric mass-exchange coefficient. Assurance of $k_L a$ is accomplished by recording the framework reaction following a change of the harmony oxygen fixation.

Integrating the outflow of the past condition yields Eq. 11.5:

$$\ln \frac{\left(C^* - C_2\right)}{\left(C^* - C_1\right)} = k_L a \times \left(t_2 - t_1\right) \tag{11.7}$$

Here, C_1 is the convergence of oxygen at time t_1. For example, in a framework that focuses on dispersed oxygen with an underlying estimation of 0, this equation progresses toward becoming an instance that declines the broken-down oxygen fixation:

$$\ln\left(1 - \frac{C_L}{C^*}\right) = -k_L a \times t \tag{11.8}$$

For the case of an issue that decreases the dispersed oxygen fixation, the equation moves toward becoming

$$\ln\left(\frac{C_{L0}}{C_L}\right) = k_L a \times t \tag{11.9}$$

Here, $k_L a$ is affected by numerous factors. Variables incorporate everything from a bioreactor's size and configuration to the sparging of gas, blending, cell line, media sort, temperature, pH, salt substance, and antifoaming specialists (2). When one of those elements changes, the elements of the bioprocess, including $k_L a$, also change. Given such a broad weakness, it is not surprising that genuine in-preparation $k_L a$ estimation has been constrained. Researchers have depended to a large degree on the implicit usefulness of the bioprocesser to maintain an appropriate oxygen stream rate.

Because k_La estimations include checking levels of dispersed oxygen following a framework issue, the outcomes can be affected by the reaction time (or speed) of a sensor making those judgments. Sensors with reaction times (τr) on the request of the main demand time steady of the mass-exchange ($1/k_La$) require careful treatment of their information to account for the time delays in readings reported by the oxygen sensor.

For the impact of this effect to be negligible, a general guideline is that the sensor's reaction time must be less than one-tenth of the time required for mass exchange. If a sensor's reaction time does not meet this prerequisite, the information must be treated with Eq. 11.10:

$$C_{\text{me}}(t) = C^* + \frac{C^* - C_0}{1 - \tau_r k_L a}\left[\tau_r k_L a e^{\frac{-t}{\tau_r}} - e^{(-k_L a)}\right] \tag{11.10}$$

in which $C_{\text{me}}(t)$ is the deliberate estimation of oxygen at time t. That condition cannot be linearized, so the nonlinear fitting of the observed reaction information is required to estimate k_La.

The estimation of k_La is interesting for both the size and setup of a reactor vessel. Although some observationally inferred articulations have been defined for k_La in non-Newtonian liquids, there is not an endless supply of conditions that can influence the outcomes. Similarly, anticipating k_La is impractical, and k_La estimations should be performed only for bioreactors.

The estimation of k_La rate constants can be calculated for any reactor vessel, from a shaker carafe to the most advanced single-use bioreactor. However, the applicability of k_La to a wide range of elements likewise changes the most ideal approach to quantify it. Correspondingly, distinctions in dispensable bioreactor development and working technique indicate a need to change the general guidelines for k_La estimation. This is not very troublesome, but rather is essentially an impression of contrasts in bioreactors.

The estimation of k_La is similar for both O_2 exchange from the air to the broth and CO_2 from it. For oxygen exchange, the driving force is the distinction between the oxygen focus around the bubbles and that in the broth, which should be held over the basic dO_2 throughout the fermenter for the duration of the procedure. Likewise, the dCO_2 must be stored underneath, which will prompt a decrease in fermentation rate or efficiency.

In low-thickness frameworks, it has been reported (Pangarkar 2015) that k_La is subject to only two parameters: the aggregate mean specific energy dissemination rate forced on the framework, $\left(\overline{\varepsilon_T}\right)_g$ (W/kg) and the shallow air speed vs (m/s), which is equal to (vvm/60) (volume of broth) / (X – the sectional area of the bioreactor). $\left(\overline{\varepsilon_T}\right)_g$ and v_S should together be adequate to deliver the vital k_La, where

$$k_L a = A\left(\overline{\varepsilon_T}\right)_g^{\alpha} \left(v_S\right)^{\beta} \tag{11.11}$$

This equation is independent of the impeller type and scale, and α and β are generally approximately 0.5 ± 0.1 regardless of the fluid. However, A is largely dependent on the development medium characteristics (Pangarkar 2015; Kroschwitz 2007). An expansion of antifoam that decreases $k_L a$ or salts that increase $k_L a$ may prompt a 20 overlay distinction in $k_L a$ for similar estimations of $\left(\overline{\varepsilon_T}\right)_g$ and v_s. Regular estimations of $\left(\overline{\varepsilon_T}\right)_g$ are approximately 5 W/kg. For the air flow rate, there is approximately 1 volume of air for each volume of development medium (vvm). Because the estimation of $k_L a$ is comparable for both O_2 and CO_2 exchange, if scale-up is implemented at a steady vvm (or near it), the driving force for the movement of O_2 in and CO_2 out will remain basically consistent over the scales. In this scenario, because vvm scales with the fermenter volume and *vs* scales with its cross-sectional zone, *vs* is incremental. There is some deliberation about whether $\left(\overline{\varepsilon_T}\right)_g$ should incorporate the influence of the sparged air (≈*vsg*, where *g* is the acceleration from gravity, 9.81 m^2/s), which becomes significant on scale-up at steady vvm. This approach should also solve issues with high dCO_2 on scale-up (Nienow 2006).

Modernization of Fermenters Using Computer Simulations

The scale-up of bioreactors is necessary to increase the amount and efficiency of manufacturing. However, the design, development, and assessment of bioreactors for large-scale production are expensive and time-consuming. Some basic restricting variables include fluid mechanics, such as incomplete mixing, supplement and oxygen circulation, and mass exchange. The yield of biomolecules in an aerobic bioreactor shifts significantly with general oxygen mass exchange ($K_L a$). To increase the yield of bioreactor operations, it is common to increase oxygen admission. However, this expanding gas stream rate also causes issues. First, it causes extreme shear force on biomolecules and cells, possibly harming them (although evidence for this in the literature is sparse). Second, extreme oxygen promotes frothing, which influences reaction volumes and thus affects efficiency. A bioreactor's operations and its surroundings are frequently determined by exploratory work, which is costly and delays production. (Biomolecular yields also rely upon biology and biochemistry; however, these topics are outside the scope of this chapter.)

Computational liquid dynamics (CLD) can be used to recreate and optimize blending, gas hold-up and mass exchange coefficients, and the dispersion of gasses inside bioreactors. Also, CLD can be used to simulate upstream operation steps, such as cleaning-in-place exercises, sanitization cycles, and the area and rates for including food, buffers, and nutrients. CLD can help to improve a bioreactor procedure by evaluating shear stresses, stream fields, and mass-exchange qualities. Downstream procedures, such as the scale-up of columns for chromatography, can be determined using CLD. This section concentrates on the hydrodynamic and blending impacts during the scale-up of bioreactors and combined multiphase liquid-gas hydrodynamics, as well as the transfer of oxygen in conveying and mixed tank bioreactors.

Impact of Mixing

Almost all bioreactors used in industry are mixed tank reactors that include liquid blending. Vessel arrangements and impellers can affect item quality, yield, and purity. Often, a blended tank configuration results in incomplete blending. Examinations with CLD and blending speculations can provide insight into bioreactor scale-up. CLD can demonstrate blending impacts, including a forecast of mix times, control numbers, turbulence amounts, and shear amounts. CLD also tells the time-history of turbulence and shear amounts accomplished by cells in a bioreactor. These insights can be a important component for scaling-up bioreactors alongside the customary tip-speed rules or power numbers. For example, during scale-up or downsizing investigations, these forecasts can help to determine impeller rpm speeds, types, and locations. Most organizations lack the adaptability to change impellers or purchase new gear, so they must select the correct reactor vessel from the available reactors inside the facility. CLD expectations can be regularly reviewed with ease through small-scale tests, such as downsize runs. Because the fundamental principles do not vary with scale, these models can apply over different scales (Wittmann et al. 2017; Paul et al. 2004; Baltz et al. 2010).

Mass Transfer Liquid-Gas Impact

In large-scale aerobic bioreactors, oxygen is typically a constraining component because of its low dissolvability into culture media. Dispersed oxygen concentrations are required to meet basic oxygen requests by microorganisms (Flickinger 2013; McDuffie 2013; Nielsen et al. 2012). The rate of air decreases below a dispersed oxygen concentration of 0.005–0.02 mM/L for most life forms. This should be unsurprising over a range of scales that can span four levels of magnitude between laboratory research and production levels. In mammalian cell-culture bioreactors, CO_2 generation and outcome mass exchange are also of importance.

The modelling perspective portrayed here for oxygen exchange is sufficiently general to be applied when investigating CO_2 development. The mixing consistency is basic for oxygen circulation in a bioreactor; however, different variables affecting mass exchange include bubble size dispersion, dispersed oxygen concentration, and liquid pressure. Bubble size determines the accessible interfacial ranges for liquid-gas mass exchange and is influenced by parameters such as turbulence, shear rate, and buoyancy. Bubbles disperse and join together due to their associations with turbulent swirls, which provides conveyance for a range of bubble sizes. When bioreactors are scaled up from the research laboratory to production size, their design must meet both oxygen conveyance and oxygen mass exchange requirements. Therefore, a precise predication of bubble size conveyance is required to determine the stream attributes and interfacial zones for heat and mass exchange computations (Nielsen et al. 2012; McDuffie 2013).

Planning

A bioreactor outline and scale-up comprehension can be increased through orderly modelling trials that start with blending investigations using CLD and blending speculations. In an organized display approach, blending can be considered and later consolidated with multiphase stream flow using bubble connections and size distribution to foresee gas–liquid mass exchange. A summary of approaches to address foreseeable oxygen exchange for bioreactors is presented here, with testing approval for two contextual analyses. An arrangement of a populace adjustment equation for bubble number thickness is combined with CLD estimations to anticipate bubble measure distribution. For the two cases considered here, the gas hold-up and fluid volumetric mass exchange coefficients were both observed to be in complete concurrence with trial results. The advantages of an appropriate plan include monitoring hazards during scale-up and decreasing downtime.

Configuration of Fermentation Vessels

This section explores the engineering applications and aspects of a range of fermentation technologies, as well as the advantages and drawbacks of the individual technologies. The fermentation techniques discussed here include surface/tray, trickle-bed setups, continuous, air-lift loop, batch, submerged, and semi-continuous. As mentioned previously, almost all of the technologies have broad applicability.

Fermentation in Batch Bioreactors

Many fermentations are batch-wise (Fig. 11.12). A batch fermenter with a stirred system is an upright, closed, tube-shaped vat with baffles connected to the wall, a water coat or heating/cooling convolution, a gadget for persuasive air circulation (called a sparger), a mechanical stirrer that generally has two or more impellers, a way to introduce nutrients and organisms, a way to collect samples, and outlets for releasing gasses. Today’s fermenters are often computerized and generally have methods for constantly observing, recording, or controlling pH, oxidation/reduction

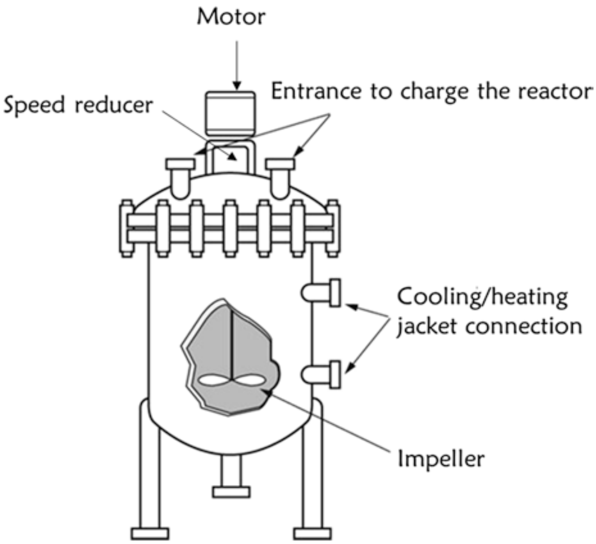


Fig. 11.12 Batch reactor diagram

potential, dissolved oxygen, CO_2 and O_2 effluent, and fermentation compounds. However, a fermenter without all of these gadgets can still be operated manually.

The primary part of batch fermentation is usually sterilization. Then, the disinfected medium is injected with microbes that were selected to attain a particular outcome. Throughout a vigorous reaction, cells, substratum, vitamins, nutrient salts, and aggregations of the item fluctuate with time. The correct admixture maintains the variations in characteristics and temperature at satisfactory levels. To achieve aerobic growth, the environment is aerated to produce a never-ending flow of a chemical element. Frothy byproducts (similar to CO_2) are removed, and gas-shifting and aeration procedures occur virtually ceaselessly. An alkali or acid is intercalary if the pH scale must be governed. To maintain adequate frothing levels, antifoaming agents may also be intercalary if required by a foam-sensing element.

A large chrome-steel batch bioreactor for manufacturing beer is shown in Fig. 11.1. (A batch bioreactor is analogous to the fermenter showed in Fig. 11.3, although without the reprocess loop.) One of the main types of batch systems is the receptacle bioreactor, which is used for economical aerobic bioreactions for items such as acids and antibiotics. In this system, the plates are filled with the medium as well as the microbes; furthermore, the airflow gives rise to the fermentation, during which the exhalation gas is released (Najafpour 2006; Cinar et al. 2003). Once the fermentation is complete, the final result is far from the plates. Thus, this technique is ineffective for manufacturing large commercial quantities. It drops rapidly to the edge and exposes the immersing tank systems, which are planned to hold considerably higher capacities.

Generally, batch fermentation systems have a variety of benefits, including the following: smaller risk of poisoning or cell change, because of a comparatively transient extension amount; less capital risk than continual procedures for a similar fermenter volume; additional adaptability for a variety of objects and biotic systems; and greater product transformation levels because of confidence in the development quantity (Cinar et al. 2003). The drawbacks include the following: lower efficiency levels because of the time required for cooling, sterilizing, heating, filling, evacuating, and cleaning the fermenter; multiple target instrumentations because of the recurring need for sterilization; greater cost associated with the need for many subcultures for immunization; higher prices for task and/or method management because the method is not continuous; and the risks associated with large-scale industrial sanitation, such as possible contact with unhealthy microbes or toxins. Typical implementation problems for batch fermenters include the following: a need to create the item with a reduced risk of pollution or microbe mutation; operations in which only very small quantities of items are created; a method that utilizes one reactor to create a variety of products; and techniques during in semi-continuous or batch item break-up is suitable.

Tubular Fermenters

The tube-shaped fermenter received its name because it looks like a tube. Tubular fermenters are usually continuous unperturbed fermenters in which the reactants progress in a predictable route. Reactants enter from one side and evacuate from the other side, with no stirring. Because of the lack of stirring, there is a progressive reduction in the concentration of substrate between the inlet point and the outlet; at the same time, the product expands similarly.

Fermentation in Continuous Bioreactors

A continuous mixed fermenter is basically like a batch fermenter, with a difference in the inlet and outlet media. An explanation of the attributes of continuous fermentation is very involved. A culture environment, which is either aseptic or contains microbes, is constantly added into the fermenter to maintain a consistent state (Chen 2013; El-Mansi et al. 2011). The product is also removed constantly from the fermenter. Reaction factors and constraint factors are stable, allowing for a consistent state inside the fermenter (El-Mansi et al. 2011). The outcome is continuous profitability and yield.

This setup has a number of potential advantages, including the following: expanded possibility for procedure automatization; lower task cost because of robotization; reduced unprofitable time associate with purging, filling, and sanitizing the reactor; reliable item quality because of consistent operating conditions; reduced danger to staff because of mechanization; and decreased stress on equipment from sanitizing. The disadvantages of continuous fermenters include the following: limited adaptability, even when only slight varieties in the procedure are needed (e.g., throughput, environment, oxygen temperature, fixation); obligatory consistency of crude materials to guarantee that the procedure remains continuous; larger capital investment in the charge and mechanization of the vessel; greater costs for the continuous sanitation of the environment; higher operating costs with the nonstop recharging of insoluble, strong substratum, such as straw; and greater danger of tainting and cell transformation because of the moderately short development timeframe. A continuous bioreaction is sometimes used for procedures with high-volume generation; techniques using gas, fluid, or dissolvable substrates; and methods including microbes with high change dependability. Typical finished products include vinegar, processed wastewater, and baker's yeast.

Fermentation Using Semi-continuous Bioreactors

Semi-continuous bioreactors use a hybrid method of continuous and batch functioning in a variety of procedures. Most commonly, the fermentation begins in a batch system, until the development-restricting substrate has taken over (Lim and Shin 2013; El-Mansi et al. 2011). At that point, the substratum is bolstered to the fermenter as indicated (batch) or is maintained by an increased culture time (continuous). For auxiliary metabolite generation, in which cell development and item creation regularly happen in distinct stages, the substrate is usually included at a predefined rate. Like batch fermenters, semi-continuous fermenters do not stop operating.

This setup has a number of advantages, including the following: increase yield, resulting from a very defined development period during which no cells are added or removed; more opportunities to optimize the environmental conditions of the microbes concerning the period of development or generation and maturation of the culture; and almost stationary functioning, which is vital for transforming organisms and those at risk for tainting (El-Mansi et al. 2011). The disadvantages include the following: lower efficiency levels because of the time-consuming need for filling, warming, sanitizing, cooling, discharging, and cleaning the fermenter; greater labor costs; and potentially powerful process control requirements for the procedure. Semi-continuous fermenters are typically used when continuous techniques are not feasible, such as when slight transformation or pollution of the organism occurs. These fermenters are also used when batch techniques do not provide the desired efficiency levels.

Mixed Tank: Submerged Bioreactors

The most well-known oxygen-consuming bioreactor being used today is the mixed tank reactor (El-Mansi et al. 2011; Saxena 2015; Inamdar 2012). Ideal for large-scale implementations, this bioreactor requires low capital and has low task expenses. For research center investigations with small volumes, the stirring vessel

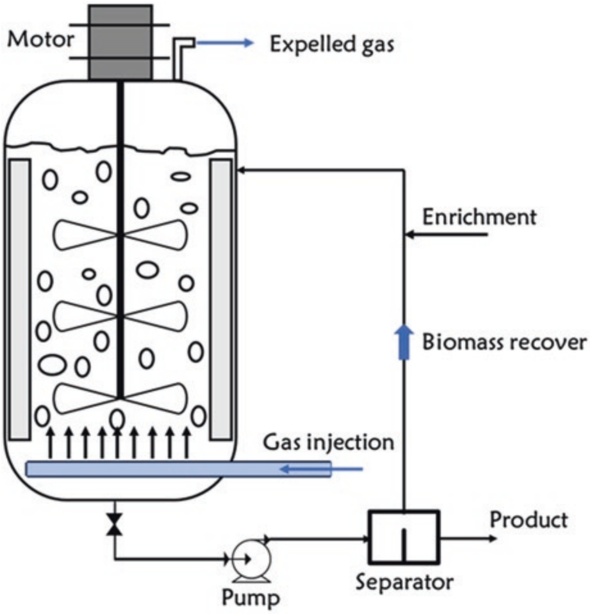


Fig. 11.13 Stirred tank – submerged bioreactors

is normally contained in glass. A stainless-steel vessel is used for large-scale (industrial) implementations. The height-to-diameter ratio of the vessel can vary based on the evacuation requirements.

The operating standards of a mixed tank bioreactor are generally basic. As shown in Fig. 11.13, the inoculum and clean medium is brought into a disinfected tank, and the air injection regularly infiltrates at the base. For ideal stirring, the tank highlights an instigator system as well as confounds, which have a whirlpool effect that could obstruct legitimate stirring. In the beginning of the procedure, warm water may be passed through the baffles to warm up the equipment; afterward, cold water could be circled within them to protect against overheating. The number of baffles commonly ranges from 4 to 8. As the fermentation advances, the bubbles delivered by the air injection are isolated by the fermenter as they progress upward.

A variety of fermenters are currently in use. The most widely recognized is the four-bladed circle turbine. More modern designs have 12 or 18 sharp edges, or sunken ones, which seem to enhance the hydrodynamics. At the highest point of the tank, gas fumes are released and the item streams down, where it is removed from the tank. In a constant-stream mixed tank reactor, the substrate is continuously sustained in the framework and the item is consistently removed and isolated, with the substrate returned once again into the tank for reuse. Likewise, with uniform chemical reactors, bioreactors can be used in parallel or series with controlled reuse streams.

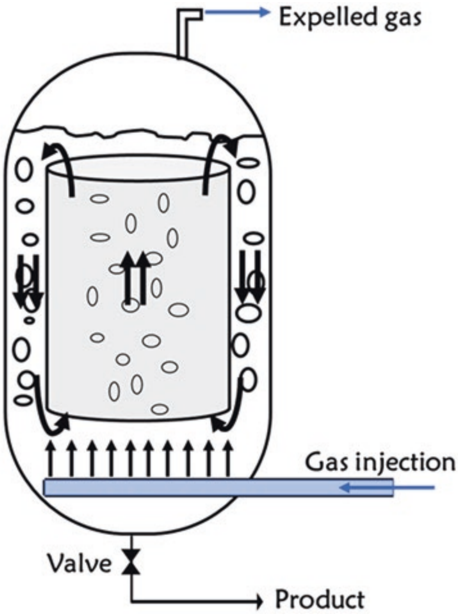


Fig. 11.14 Airlift bioreactor

Airlift Reactor Equipment

In an airlift reactor (also called a tower reactor), the bubble column includes an assembly conduit (Saxena 2015). Various airlift reactors are being used today. Air is commonly sent via an irrigator into the base of a median draft tube, which guides the flow of air and the milieu (Fig. 11.14). Gas goes up the tube, shaping bubbles, and gas fumes separates at the highest point of the section. Degassed fluid then descends, and the item is depleted from the tank. The tube is intended to act as an interior heat exchanger; otherwise, a heat exchanger could be attached to an inward flow loop (Saxena 2015).

The airlift approach has a few benefits over other customary bioreactors, such as the classical fermenter. For example, it has a straightforward design with no moving parts or fermenter shaft seals, which requires less maintenance, has less danger of deformities, and needs less demanding sanitation. Furthermore, it has a smaller shear rate for more prominent adaptability, the ability to be used to develop both animal and plant cells; effective gas-stage withdrawal; a substantial and defined interfacial contact zone with less energy input; a highly controlled stream and productive stirring; well-characterized residence time for all stages; expanded mass-exchange because of the improved oxygen solvency accomplished in extensive tanks with more noteworthy pressures; the possibility for large-volume tanks and thus expanded yield; and greater heat release compared with customary mixed tanks.

The fundamental weaknesses of this approach include the following: higher start-up capital investments because of the large-scale procedures; more prominent air throughput and high pressures required, especially for large-scale operation; limited abrasion with an ideal pressure driven diameter for the ascender and downcomer; limited productivity of gas pressure; great difficulty in maintaining steady levels of substratum, supplements, and oxygen with the microbes circling through the bioreactor and states transforming; and wasteful gas/fluid detachment while frothing occurs. However, these disadvantages can and should be controlled for when planning airlift frameworks. For example, if just a single area serves as the sustain source, the microbe would encounter endless cycles of high development followed by starvation; this would cause undesirable results, low yields, and high death rates. A design with numerous sustain points would eliminate this hazard, particularly for large-scale operations. Similar dangers are found with a solitary passage point for oxygen; oxygen should be transmitted at different places inside the vessel, with most of the air entering at the base to circle the liquid through the reactor.

External-Loop Airlift Reactors

Another kind of airlift technology is the airlift external-loop reactor system (AELR), which is generally used for a batch system. The AELR illustrates a different perspective, where the downcomer and vessel are actually higher than they

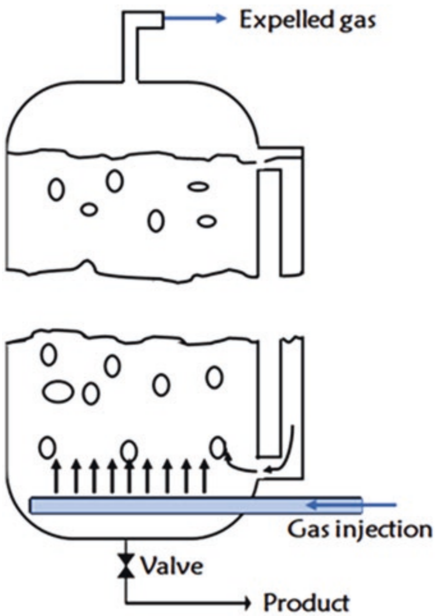


Fig. 11.15 External-loop airlift bioreactor

appear for the specific extended diameter (Fig. 11.15). As a variation of the airlift technique, the AELR uses a prompted course to coordinate air and fluid throughout the vessel. This approach comprises a riser and an outer downcomer, which are associated with the base and the top, respectively (Saxena 2015). As infused air at the base of the riser makes gas bubbles that start to ascend through the principal tank, gas fumes separate at the top; the subsequent dense solution drops through the downcomer.

The AELR has a few advantages over the classic airlift technique, including the following: effectual heat exchange and productive temperature control; a small abrasion with an ideal pressure-driven width for both the downcomer and riser; a highly defined time for the individual segments of the AELR; an expanded open door for estimation and control in the riser and the downcomer; and free control of the gas input-rate and fluid speed by a throttling gadget in the riser and downcomer.

Fluidized Bed Fermenters

Fluidized bed fermenters are similar to tube-shaped fermenters. In both continuous mixed fermenters and tube-shaped fermenters, there is a genuine threat of the microorganisms being drained. The fluidized bed reactor addresses this issue as a hybrid of the mixed tank and tubular tank setups. The organisms in a fluidized bed fermenter are maintained in suspension by a circulating rate whose power adjusts to gravity. If the movement was smaller, the bed would stay settled. If the circulating rate was at a higher speed than the mass of the organisms, elutriation would occur, with the organisms being drained from the fermenter.

Anaerobic Bioreactions

Anaerobic bioreactions occur within a number of setups, such as ethanol creation, winemaking, lager blending, and wastewater treatment. Advances have been reported for wine, ethanol, and lager creation due to product changes and decreased assembly costs. Consistent bioreactors have been marketed for brewing, but group fermenters continue to receive capital investments. Waste treatment is a field that has developed because of very useful innovations in setup; however, not as much consideration has been given to creating more modern wastewater treatment systems.

Conclusion

Today, fermentation operations are under pressure from demands for changes to product ranges and the scale of manufacturing. This can be ascribed to a variety of factors, including large-scale bioprocesses and improved fermentation techniques, which have prompted the advancement of new fermented foods. To drive these procedures—both new and old—a comprehensive understanding of food fermentation is required. Policymaking has also played a part in hastening improvements within this industry. For example, modern policies have expanded research and development investments for new equipment designs, and in addition to guiding the directions, standards, and licensed innovations that address the issues resulting from increased production. Thus, this chapter presented an outline of various aspects of modern bioreactors and innovations in the industry. The nutritional status of fermented foods can likewise be enhanced by the informed selection of maturing organisms with respect to the human diet and gastrointestinal microbiota. In this regard, fermented food could be viewed as an augmentation of food absorption and aging procedures; thus, it could be designed to provide valuable benefits to human health and well-being. Another planned technique to increase the metabolic efficiency of bioprocesses is the use of appropriately controlled ultrasonication, which can be used at the biocatalyst level (cells and catalysts) to improve their capacity and sonobioreactor execution.

References

Adams RD (1985) Vinegar. In: Wood B (ed) Microbiology of fermented foods. Elsevier, Philadelphia, pp 1–49

Al-Rubeai M (2015) Animal Cell Culture. Cell Engineering, vol 9. Springer International Publishing. https://doi.org/10.1007/978-3-319-10320-4

Anon (2008) Whitbread's Brewery - Incorporating The Brewer's Art (Paperback). Read Books, United Kingdom

Baltz RH, Demain AL, Davies JE (2010) Manual of industrial microbiology and biotechnology. ASM Press, Washington, DC

Briggs DE, Boulton CA, Brookes PA, Stevens R (2004) Brewing science and practice. Woodhead Publishing Limited and CRC Press LLC, Cambridge

Bylund G (1995) Cultured milk products. In: Dairy processing handbook. Tetra Pak Processing Systems AB, Lund, pp 241–262

Campbell-Platt G (1994) Fermented foods – a world perspective. Food Res Int 27:253–257

Chandan RC (2014) Dairy – fermented products. In: Clark S, Jung S, Lamsal B (eds) Food processing: principles and applications, 2nd edn. Wiley, Chichester, pp 405–436

Chen H (2013) Modern solid state fermentation: theory and practice. Springer Netherlands, Dordrecht

Cinar A, Parulekar SJ, Undey C, Birol G (2003) Batch fermentation: modeling: monitoring, and control. CRC Press, New York

Clark S, Costello M, Drake MA, Bodyfelt F (2009) The sensory evaluation of dairy products. Springer, New York

Corrieu G, Monnet C, Sepulchre AM (2005) Use of strains of Streptococcus thermophilus which are incapable of hydrolyzing urea in dairy products. USA Patent
Demeyer D, Stahnke L (2002) Quality control of fermented meat products. In: John K, David L (eds) Meat processing: improving quality. Woodhead, Cambridge, UK, pp 359–393
Doran PM (2013) Bioprocess Engineering Principles. Engineering professional collection. Waltham, M. A. & Academic Press, 225 Wyman Street, Waltham, MA 02451, USA
El-Mansi EMT, Bryce CFA, Demain AL, Allman AR (2011) Fermentation Microbiology and Biotechnology, Third Edition. CRC Press, Taylor & Francis group, Boca Raton
Eva H, de la Hoz L, Juan AO (1997) Contribution of microbial and meat endogenous enzymes to the lipolysis of dry fermented sausages. J Agric Food Chem 45(8):2989–2995
Flickinger MC (2013) Upstream industrial biotechnology, 2 volume set. Wiley, New York
Gänzle MG, Ehmann M, Hammes WP (1998) Modeling of growth of Lactobacillus sanfranciscensis and Candida milleri in response to process parameters of sourdough fermentation. Appl Environ Microbiol 64(7):2616–2623
Gupta BS, Ibrahim S (2013) Mixing and crystallization: selected papers from the international conference on mixing and crystallization held at Tioman Island, Malaysia in April 1998. Springer Netherlands, Dordrecht
Hills M (2014) Cider Vinegar. 2nd edn. Sheldon Press, Great Britain
Hutkins RW (2006) Microbiology and technology of fermented foods. Blackwell Publishing, Ames
Inamdar STA (2012) Biochemical engineering: principles and concepts. PHI Learning, New Delhi
Inui M, Vertes AA, Yukawa H (2010) Advanced fermentation technologies. In: Vertes AA, Qureshi N, Blashek HP, Yukawa H (eds) Biomass to biofuels. Blackwell Publishing, Ltd, Oxford, pp 311–330
Kroschwitz JI (2007) Kirk-Othmer encyclopedia of chemical technology, vol 25, 5th edn. Wiley, Hoboken
Laskin AI, Sariaslani S, Gadd GM (2011) Advances in Applied Microbiology, vol 77. First edn. Academic Press, San Diego, USA
Leistner F (1992) The essentials of producing stable and safe raw fermented sausages. In: Smulders FJM, Toldrá F, Flores J, Prieto M (eds) News technologies for meat and meat products. ECCEAMST, Audet Tud – schreen, B.V., Utrecht, pp 1–19
Lim HC, Shin HS (2013) Fed-batch cultures: principles and applications of semi-batch bioreactors. Cambridge University Press, Cambridge
McDuffie NG (2013) Bioreactor design fundamentals. Elsevier Science
Mulchandani A, Bassi AS, Nguyen A (1995) Tetrathiafulvalene-mediated biosensor for L-lactate in dairy products. J Food Sci 60:74–78
Najafpour G (2006) Biochemical Engineering and Biotechnology. First edn. Elsevier Science, New York
Nielsen J, Villadsen J, Lidén G (2012) Bioreaction Engineering Principles. Second edn. Springer USA
Nienow AW (2006) Reactor engineering in large-scale animal cell culture. Cytotechnology 50:9–33
Nienow AW, Edwards MF, Harnby N (1997) Mixing in the Process Industries. Second edn. Butterworth-Heinemann, Johannesburg
Nienow AW, McLeod G, Hewitt CJ (2010) Studies supporting the use of mechanical mixing in large scale beer fermentations. Biotechnol Lett 32(5):623–633. https://doi.org/10.1007/s10529-010-0213-0
Nienow AW, Nordkvist M, Boulton CA (2011) Scale-down/scale-up studies leading to improved commercial beer fermentation. Biotechnol J 6(8):911–925. https://doi.org/10.1002/biot.201000414
Pangarkar VG (2015) Design of multiphase reactors. Wiley, Hoboken
Park YW (2009) Bioactive components in milk and dairy products. Wiley, Ames

Paul EL, Atiemo-Obeng VA, Kresta SM (2004) Handbook of industrial mixing: science and practice. Wiley, Hoboken

Saxena S (2015) Applied microbiology. Springer India, New Delhi

Tamime AY (2008) Structure of dairy products. Wiley, New York

Toldrá F (2008) Meat biotechnology. Springer, New York

Toldrá F (2010) Handbook of meat processing. Wiley, Oxford

Toldrá F, Sanz Y, Flores M (2001) Meat fermentation technology. In: Hui YH, Nip WK, Rogers RW, Young O (eds) Meat science and applications. Marcel Dekker, New York, pp 537–563

Toldrá F, Hui YH, Astiasaran I, Sebranek J, Talon R (2014) Handbook of Fermented Meat and Poultry. Second edn. Wiley, Oxford, UK

Watanabe K, Fujimoto J, Sasamoto M, Dugersuren J, Tumursuh T, Demberel S (2008) Diversity of lactic acid bacteria and yeasts in Airag and Tarag, traditional fermented milk products of Mongolia. World J Microbiol Biotechnol 24:1313–1325

Wittmann C, Liao JC, Lee SY, Nielsen J, Stephanopoulos G (2017) Industrial Biotechnology: Products and Processes, vol 4. Advanced Biotechnology, First edn. Wiley-VCH, Germany

Chapter 12
Advances in Genetic Engineering for Higher Production and Quality Improvement of Food and Beverages

Aly Farag El Sheikha

Abstract Genetic engineering promises to bring important and rapid leaps in food production. It will affect all steps of the production chain, from farm to final food processing. As a biotechnological practice, it has the potential to be used as an effective tool to address the various problems in food and society. Nowadays, our production-to-consumption food system is complex, and consumer needs are growing toward food which is safe, nutritious, abundant, diverse, and less costly. Therefore, scientific and technological advancements must be accelerated and applied in developed and developing countries alike if we are to feed a growing world population. This chapter deals with an analytical perspective on the food applications of genetic engineering that made possible the modern production-to-consumption food system capable of feeding nearly seven billion people, and it also discusses the benefits of this promising biotechnological tool to enhance the food supply in terms of quantity and quality even further and to increase the health and wellness of the growing global population. However, as with any new technology, careful consideration of the effects of employing these tools is necessary to ensure that the result will be a net benefit to humanity. Recent controversies about genetically engineered foods have highlighted the need to allay consumer fears by applying the reliable techniques of traceability and also providing more experimental evidence and sound scientific judgment to assess the risks versus benefits.

Keywords Genetic engineering (GE) · Genetically modified foods (GMFs) · GE benefits, GE potential risks · Feeding worldwide, Traceability, PCR-based techniques

---

A. F. El Sheikha (✉)
Department of Biology, McMaster University, Hamilton, Ontario, Canada

Department of Food Science and Technology, Faculty of Agriculture, Minufiya University, Minufiya Government, Shibin El Kom, Egypt

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_12

Related Important Terms

Biotechnology

Biotechnology is a broad term that applies to the use of living organisms and covers techniques that range from simple to sophisticated (Zhao and McDaniel 2005). According to the Secretariat of the Convention on Biological Diversity (2000), biotechnology is defined as (1) the application of in vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and the direct injection of nucleic acid into cells or organelles, or (2) the fusion of cells beyond the taxonomic family that overcomes natural physiological reproductive or recombination barriers and is not a technique used in traditional breeding and selection.

Since the dawn of history, man has sought to crossbreed many related species of plants and animals to develop new or hybrid varieties with useful traits, such as increasing productivity and improving quality. Traditional crossbreeding produces changes in the genetic makeup of a plant or animal which consume times as it is necessary to breed numerous generations in order to not only obtain the desired trait but also remove several unwanted characteristics (Giuseppe et al. 2010).

Food Biotechnology

Food biotechnology is defined as the application of biological techniques to food derived from plants, animals, and microorganisms with the target of improving the food attributes in terms of quantity, quality, and safety and also the economics of processing and production (Habibi-Najafi 2006).

Genetic Engineering

Genetic engineering (GE) is the artificial modifications of the organism's genetic composition that involving the transfer of specific genes (expressed by traits), from one organism into a plant or animal of an entirely different species. The organism resulting after gene transferring is called genetically modified organism (GMO) (GRACE Communications Foundation 2017).

In genetic engineering, genes can be transferred from completely different species inserted into one another as one of the advantages of genetic engineering which makes it different from traditional crossbreeding, where genes can only be exchanged between closely related species (GRACE Communications Foundation 2017).

Genes

The cell is the building unit of the organism. Each cell contains a nucleus, and inside the nucleus are strands of DNA (deoxyribonucleic acid). Each strand of DNA is divided into small fragments called genes. These genes contain a unique set of codes that are responsible for determining the characteristics of each organism. Hence the gene could be defined as a locus (or region) of DNA which is made up of nucleotides and is the molecular unit of heredity (Slack 2014). These genes make up diverse DNA sequences called genotypes. Genotypes along with environmental factors define what the phenotypes will be (GRACE Communications Foundation 2017).

Genetically Modified Organisms (GMOs)

Genetically modified organisms (GMOs) can be defined as organisms (i.e., plants, animals, or microorganisms) in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination. The technology is often called "modern biotechnology" or "gene technology," sometimes also "recombinant DNA technology" or "genetic engineering." It allows selected individual genes to be transferred from one organism into another, also between nonrelated species. Foods produced from or using GM organisms are often referred to as GM foods (WHO 2014).

Genetically Modified Foods (GMFs)

Genetically modified foods (GMFs) are foods derived from organisms whose genetic material (DNA) has been modified in a way that does not occur naturally, e.g., through the introduction of a gene from a different organism. Currently, available GM foods stem mostly from plants, but in the future foods derived from GM microorganisms or GM animals are likely to be introduced on the market. Most existing genetically modified crops have been developed to improve yield, through the introduction of resistance to plant diseases or of increased tolerance of herbicides (WHO 2017).

In the future, genetic modification could be aimed at altering the nutrient content of food, reducing its allergenic potential, or improving the efficiency of food production systems. All GM foods should be assessed before being allowed on the market. FAO/WHO Codex guidelines exist for risk analysis of GM food (WHO 2017).

Green Revolution

Green revolution is defined as a period in the mid- and late twentieth century (1930s–1960s) when the productivity of global agriculture increased drastically because of the use of new agricultural techniques, i.e., high-yielding varieties (HYVs) of cereals, especially dwarf wheat and rice, in association with fertilizers, synthetic herbicides and pesticides, controlled water supply, and mechanized cultivation. All together supersede the traditional technology and are to be adopted as a whole (Farmer 1986; this chapter). Dr. Norman Borlaug, widely believed to be the father of this green revolution, was awarded the Nobel Peace Prize in 1970 for his contributions to humanity (Patel 2013).

Food Quality

Food quality is a wide term involving food characteristics that are acceptable to consumers, i.e., external traits (appearance, texture, flavor) and internal traits (nutritional, chemical, physical, microbial) (Perez-Gago et al. 2006). In simple words, the product should have attributes to "satisfy the wants/needs of the consumer or conformance with the user's requirements." Quality also covers the safety and value for money (El Sheikha 2018a).

Trait

The trait is a characteristic that differentiates one plant or animal from another, such as yellow versus white corn (Tietyen et al. 2000a).

Transgenic

Transgenic is a plant or animal genetically engineered resulting from an altered or modification of genetic composition (Tietyen et al. 2000a).

Food Biotechnology: Between Past and Present

Biotechnology has a long history of use in food production and processing. For 10,000 years, fermentation as a form of biotechnology has been used to produce wine, beer, and bread (El Sheikha 2018b). Food biotechnology included many

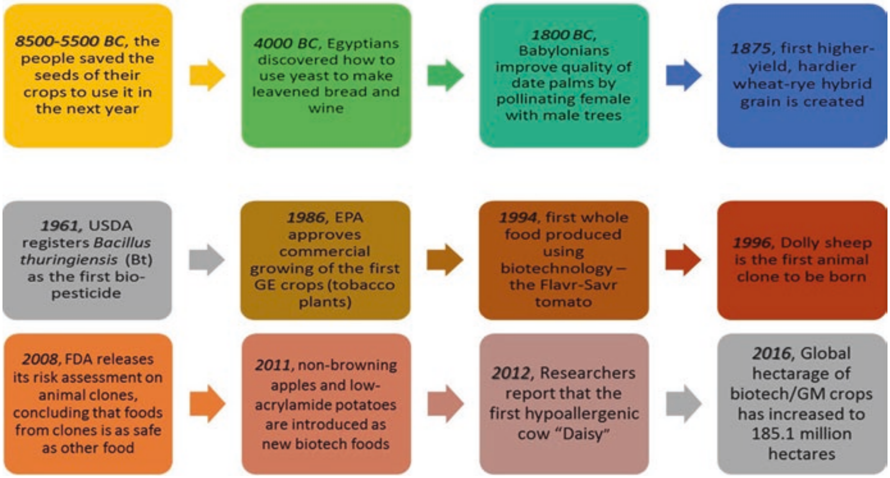


Fig. 12.1 Significant stations of food biotechnology train through history. (Sources: https://www.genome.gov/Pages/Education/GeneticTimeline; IFIC 2013; Zhang et al. 2016)

important stations throughout its history which can be traced back thousands of years. This timeline includes significant events that have led to the current use of gene technology in food technology. It also shows some predictions about future developments in the application of gene technology to food production (see Fig. 12.1).

While the origins of biotechnology can be traced to the ancient Egyptians, significant leaps in this field were made in the 1900s through today. Modern biotechnology refers to different techniques used to produce desired traits in plants, animals, or microorganisms through the use of genetic knowledge (Hsieh and Ofori 2007). Since its introduction to agriculture and food production, biotechnology has been utilized to develop new tools for improving productivity. Recently, enzymes are widely used in food industries (e.g., baking industry, fruit juice, cheese manufacturing, winemaking, brewing) to improve their characteristics in terms of flavor, texture, digestibility, safety, and nutritional value (Li et al. 2012; Hua et al. 2018; Ting et al. 2018).

Techniques Used to Genetically Modify Food

Technically, what is the meaning of genetic engineering? Simply, the genetic engineering is an insertion of new DNA sequences (usually whole gene) into an organism's chromosomal DNA. This mechanism was explained by the applied technique (Diehl 2017).

There are four key elements that are common to almost all genetic engineering procedures (Institute of Medicine and National Research Council 2004): identifying

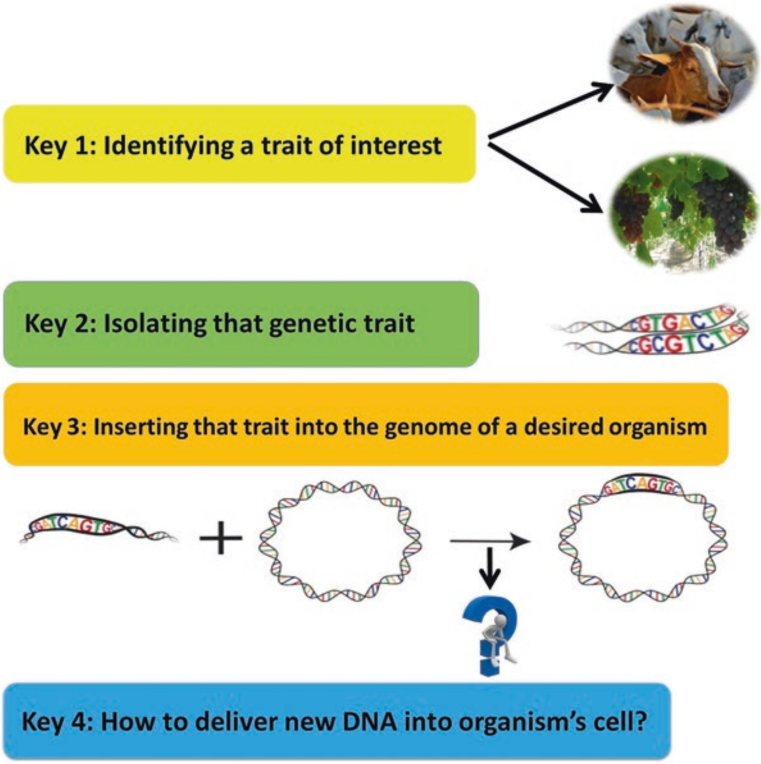


Fig. 12.2 The key elements that are common to almost all genetic engineering procedures

a trait of interest, isolating that genetic trait, inserting that trait into the genome of a desired organism, and finally, it is necessary to have a method of delivering the new DNA (i.e., promoter, new gene, and selection marker) into the organism's cells (Fig. 12.2).

Methods Used to Genetically Modify Plants

There are two main techniques that are used to modify plants genetically (Institute of Medicine and National Research Council 2004):

- Using *Agrobacterium tumefaciens* bacterium as a vector for the DNA, which has the ability to infect plants and insert DNA into its genome. This is the common method that has been used to modify broad-leaved plants (e.g., oilseed rape, sugar beet) but is now also being applied to rice and corn.

- Biolistics, i.e., particle bombardment, where the inserted DNA is coated with minute gold particles and fired into plant cells. This technique is mainly used for monocot plants (e.g., rice and maize).

Regarding the drawbacks of both GM techniques (Institute of Medicine and National Research Council 2004):

– They are not sufficiently accurate to allow the introduction of gene(s) at particular site(s) in the genome.
– There are risks to insert multiple copies of genes that may be in the forward or reverse orientation and also transferring of fragments of genes from the vector.
– The possibility of occurrence replication, deletion, and rearrangement of the plant's own genes.
– Mutations may occur at the site of insertion or be genome-wide that can cause in:

1. Disruption of endogenous genes (i.e., the plant's own genes) and their functioning
2. Negative effects on biochemical pathways such as the production of unexpected toxins or anti-nutrients
3. Silencing of genes in next generations if multiple copies exist as a result of transgene silencing

Methods Used to Genetically Modify Animals

The following techniques have been used to modify animals genetically (Institute of Medicine and National Research Council 2004):

- Microinjection (pronuclear injection): in this method, the injection of DNA into the nucleus of a single embryo cell is conducted by using a very fine needle. The incorporation of the injected DNA into the genome is random in some embryos.
- Viruses (e.g., retroviruses): which are used as vectors to introduce foreign DNA into cells because of their ability to infiltrate them. Retrovirus is a type of virus that replicates by integrating itself into the host genetic material and then copied with the host DNA.
- Embryonic stem (ES) cell culture and modification allows a much more targeted approach to genetic modification. This modification can be a transgenic gene that replaces the native one or those genes that are "knocked out" made ineffective by disruption or removal.
- Sperm-mediated transfer: in this approach, the genetically modified sperm is used as a vector for introducing new genetic material into the ova. This method is used routinely in livestock and poultry.

Recent Techniques: Genome Editing

"Genome editing" is a term used to describe a relatively new set of technologies that enable one to make precise changes in the DNA of a plant, animal, or other living organisms. For example, such technologies can be used to introduce, remove, or substitute one or more specific nucleotides at a specific site in the organism's genome (FDA 2017). Genome editing is being performed using, for example, clustered regularly interspersed short palindromic repeats (CRISPR)-associated nucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and oligonucleotide-directed mutagenesis (ODM).

In the past few years, the "CRISPR-Cas9" as a revolutionary genome-editing tool was developed (Cong et al. 2013; Ran et al. 2013), which increases the efficiency of genetic engineering and making their application to plants and animals much easier (DeMayo and Spencer 2014; Belhaj et al. 2015; Visk 2017).

Cas9 is a DNA endonuclease originally found in bacteria, where it protects the host bacteria from invading DNA molecules (e.g., viruses). The endonuclease is guided to the invading/targeting DNA by a special "guide RNA" (gRNA), whose sequence is complementary to the invading sequence to be expunged. Thus guided by the offensive sequence, Cas9 utilizes its two active sites to cleave both strands of the double-stranded DNA (Zhang et al. 2016). The newly formed DNA double-stranded breaks (DSBs) are then repaired by two different mechanisms inside cells: The "nonhomologous end joining" (NHEJ) mechanism can cause a small deletion or random DNA insertion, leading to a truncated gene or knockout, while the "homologous recombination" (HR) mechanism allows the addition of a donor DNA into the endogenous gene at the break site (Fig. 12.3).

Why Genetic Engineering?

By 2050, the global population is expected to reach nine billion people, requiring 70% more food than is produced today (FAO 2009; Godfray et al. 2010). Therefore, more yields will be required on the same land. Biotechnology is potentially the best technology to fight against the problem of food yield (Haroon and Ghazanfar 2016).

To feed the booming world population, the corresponding increase in food production is necessary. The food derived from plants and animals act as major sources of nutrition in human diet by providing certain essential amino acids and vitamins that cannot be synthesized de novo by humans. Thus, malnutrition is a complex problem for human health, causing the loss of countless lives in many countries. To be healthy, our daily diet must include ample high-quality foods with all of the essential nutrients, in addition to foods that provide health benefits beyond basic nutrition (Irfan and Datta 2017).

The challenge is to increase the food production by maintaining high productivity under various stresses as well as developing plant- and animal-based foods with

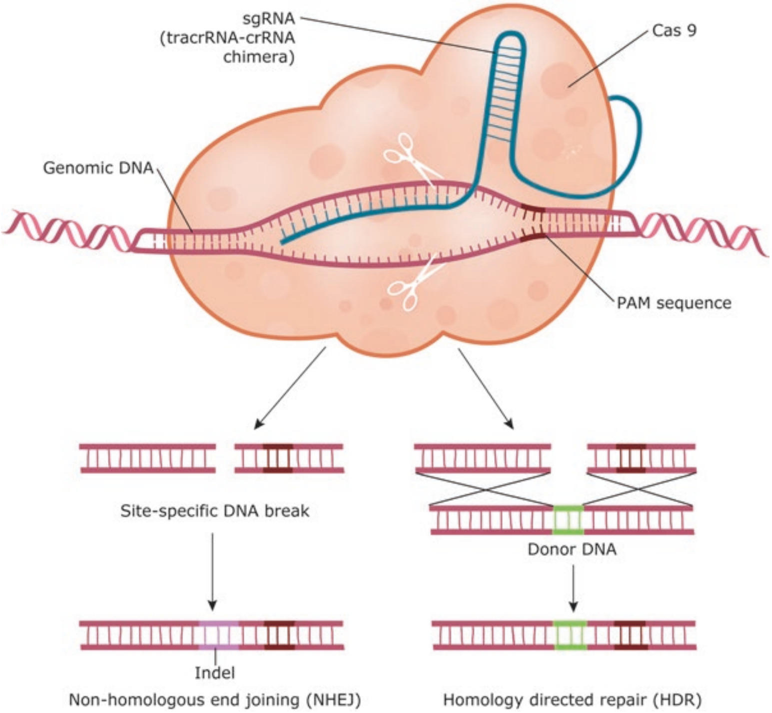


Fig. 12.3 CRISPR/Cas9-mediated genome-editing mechanism. (Source: Savić and Schwank 2016. Reproduced with permission of Elsevier)

enhanced nutritional quality. Genetically modified (GM) techniques can prove to be powerful complements to those produced by conventional methods for meeting the worldwide demand for quality foods. The modern biotechnological tools allowed the manipulation of genes (gene technology) from different sources and insertion of these genes into plants and animals to impart desirable traits in economically important foods (Irfan and Datta 2017).

What Benefits Do Consumers Expect from Food Biotechnology?

Since 2012, Americans have been increasingly confident in the benefits of using the biotechnology techniques in food, whereas 35% of consumers believing they will benefit from food biotechnology over the next five years. When those consumers are asked in 2014 what benefits they expect from food biotechnology, they say (International Food Information Council Foundation "IFIC" 2014):

– Health and nutrition
– Improved quality, taste, and variety

– Price and economic benefits
– Improved agricultural production
– Safer foods
– Reduced pesticides

In 2015, half of Americans (50%) agree that biotechnology can be one tool to help provide enough food for a growing global population. Notably, two-thirds (66%) agree that the overall healthfulness of the food and beverage is more important than the use of biotechnology in its production (IFIC 2015).

Key Benefits Related to Food Biotechnology

Four key benefits related to biotechnology help to answer the question, why genetic engineering? (IFIC 2014):

1. Food safety
2. Consumer benefits (for wholesome, nutritious, and affordable food)
3. Sustainability (for the environment, economy, and communities)
4. Feeding a hungry world (in terms of quantity and quality of staple foods to meet the needs of the growing global population)

Table 12.1 summarizes the benefits and potential risks of using the genetic engineering in food applications.

More details regarding the benefits and potential risks of applying the genetic engineering on food will be discussed in the following sections.

Table 12.1 Benefits and potential risks of using GE in food applications

Aspect	Benefits	Potential risks
Productivity	Increased crop yield	Decreased genetic diversity
Quality	Improved quality	New food allergies
	Useful in food processing	Contamination of organic crops
Safety	Provide safer food (e.g., reduced crop/food spoilage, rapid methods to monitor the pathogens, toxins, and contaminants in foods)	Increased naturally occurring toxins, antibiotic resistance transfer
Economical and social	Price and economic benefits	Social effects of new technology
Environmental	Environmental benefits (e.g., reduced pesticides, reduced fertilizer use)	Herbicide resistance, pest resistance

Genetically Engineered (GE) Crops

Global hectarage of biotech/GM crops has increased ~110-fold from 1.7 million hectares in 1996 to 185.1 million hectares in 2016 – this makes genetically modified crops the fastest adopted crop technology. The 185.1 million hectares of GM crops were grown in 26 countries, of which 19 were developing and 7 industrial countries. Developing countries grew 99.6 million hectares or 54% compared to 85.5 million hectares or 46% for industrial countries of the global biotech crop area. The four major GM crops are soybean, maize, cotton, and canola (International Service for the Acquisition of Agri-biotech Applications "ISAAA" 2016).

Of the top five countries growing biotech crops, three are developing countries (Brazil "49.1 million hectares," Argentina "23.8 million hectares," and India "10.8 million hectares"), and two are industrial (USA "72.9 million hectares" and Canada "11.6 million hectares") for a total of 168.2 million hectares, 91% of the global hectarage (Fig. 12.4, ISAAA 2016).

Genetically engineered crop supporters claim GE crops use fewer pesticides than non-GE crops when in reality GE crops can require more chemicals. This is because weeds become resistant to pesticides, leading farmers to spray more chemicals on their crops (Benbrook 2012). This would pollute the environment and expose food to higher levels of toxins which create greater food safety and environmental fears.

Until today, adequate studies have not yet been conducted to determine the effects of eating animals that have been fed genetically engineered plants, nor have sufficient research been carried out on the effects of directly consuming genetically engineered crops (e.g., corn, soy, etc.). Yet, in spite of our lack of knowledge, GE crops are widely used throughout the world as both human and animal food (GRACE Communications Foundation 2017).

Genetically Engineered (GE) Animals

A genetically engineered (GE) animal is one that contains additional or altered genetic material (e.g., recombinant DNA (rDNA)) through the use of modern biotechnological tools that's intended to give the animal a new trait or characteristic. Examples of the kind of GE animals that are being developed are provided below (FDA 2015). Various researches are being conducted to create genetically engineered farm animals, such as:

- Cattle that cannot develop the infectious prions which can cause BSE (bovine spongiform encephalopathy) (Richt et al. 2007)
- Engineered chickens so that they cannot spread avian flu virus (H5N1) to other birds (Jha 2011)
- Engineered Atlantic salmon to grow twice as fast as wild salmon (Park 2015)

Fig. 12.4 Global map of biotech crop production in 2016. (Source: ISAAA 2016, http://www.isaaa.org/resources/publications/briefs/52/executivesummary/default.asp)

On November 19, 2015, FDA approved an application related to AquAdvantage Salmon, a GE Atlantic salmon. Although these salmon will be bred in Canada and raised in Panama, food from these salmon will be imported into the USA.

Many kinds of GE animals are in development (FDA 2015), for instance:

- *Biopharm* animals are those that have undergone genetic engineering to produce particular substances, such as human insulin, for pharmaceutical use.
- *Research* animals may be engineered to make them more susceptible to particular diseases, such as cancer, in order to gain a better basic understanding of the

Table 12.2 Overview of some of the transgenic animals with potential commercial applications

Species	Transgene	Effect/goal
Cattle	Lysozyme	Milk composition
	PrP	Animal health
	α-, κ-casein	Milk composition
	Omega-3	Milk composition
	Lysostaphin	Mastitis resistance
Rabbit	Calcitonin	Osteoporosis treatment
	Erythropoietin	Anemia treatment
	Superoxide dismutase	Blood purification
	Interleukin-2	Cancer treatment
	Tissue plasmogen activator	Anti-clotting agent
	VP2, VP6	Rotavirus vaccine
Salmon	Growth hormone	Growth rate
	Lysozyme	Animal health
	wflAFP-6	Cold tolerance
Trout	Follistatin	Muscle development
Sheep	IGF-1	Wool growth
	Visna resistance	Disease resistance

Source: Adapted from Lievens et al. (2015). (Reproduced with permission of Elsevier)

disease for the development of new therapies or in order to evaluate new medical therapies.

- *Xenotransplant* animals are being engineered, so they can be used as sources for cells, tissues, or organs that can be used for transplantation into humans.
- *Companion* animals that are modified to enrich or enhance their interaction with humans (i.e., hypoallergenic pets).
- *Disease-resistant* animals may be used either for food use or biopharm applications. These animals have received modifications that make them resistant to common diseases, such as mastitis (a very painful infection of the udder) in dairy cows, or particularly deadly diseases, such as bovine spongiform encephalopathy (BSE).
- *Food* use animals have been engineered to provide healthier meat, such as pigs that contain healthy omega-3 fatty acids at levels comparable to those in fish.

Table 12.2 *includes further examples of animal genetically modifications.*

Many GE animals in development are intended to have direct benefits to consumers. For instance, biopharm animals are being developed to produce various pharmaceuticals for humans or other animals such as clotting factors, growth factors, and inhibitors used in cancer therapy, some of which cannot now be produced in sufficient quantities to meet medical needs (FDA 2015). Some GE animals are under development to produce healthier food. And other animals are under development to have indirect benefits to consumers, such as decreased environmental impact by excreting lower levels of pollutants in their wastes. By contrast, genetic engineering experiments on animals do, however, pose potential risks to health of the GE animal, food safety, and the environment. Therefore, there are risk-based reasons for FDA to require their approval (FDA 2015; GRACE Communications Foundation 2017).

Applications of Genetic Engineering to Improve Brewing, Winemaking, and Baking Industries

Yeasts are one of the most important groups of microorganisms in food industry and alcohol production. *Saccharomyces cerevisiae* is a species of budding yeasts. Yeast has proven to be very useful to humanity throughout history due to its fundamental role in the production of bread, wine, beer antioxidants, enzymes, several medical applications and recently used for pigments production (Hesham 2010). The improvement of industrial strains relied on traditionally genetic techniques (hybridization, mutagenesis, cytoduction, protoplast fusion), followed by selection for broad traits such as ethanol tolerance, fermentation capacity, fast dough fermentation, osmotolerance, rehydration tolerance, organic acid resistance (baker's strains), flocculation and carbohydrate utilization (brewer's strains), and absence of off-flavors (e.g., H_2S for wine strains). Dequin (2001) states that the main targets for strain development fall into two categories:

1. Improvement of fermentation performance and simplification of the process
2. Improvement of product quality (e.g., hygienic and organoleptic characteristics)

Traditional genetic techniques were first applied to wine yeast strains in the middle of the 1980s, in response to increasing demand for new characteristics. The new properties of wine yeast strains (e.g., flocculation properties, expressing the killer toxin) have been generated by hybridization, mutagenesis, and cytoduction (Barre et al. 1993; Dequin 2001).

Genetic Strategies Applied to Wine Yeasts as Case Study

Despite the high diversity in natural yeasts, winemakers are interested in novel strains with a combination of specific traits, which can confer a competitive advantage to the wine in terms of quality and consumer acceptance. Several attempts have been made to enhance GSH production by yeasts, although not all are suitable for winemaking (De Vero et al. 2017).

Random Mutagenesis Random mutagenesis (RM) was one of the first techniques of genetic modification ever applied. The technique is based on the application of mutagens (chemical and physical ones) in order to enhance the natural mutation rate occurring in microorganisms. Accordingly, mutations range from the minor modifications such as single-base substitutions up to DNA frameshifts and alterations of the chromosomal structure. Random mutagenesis has a limited efficiency in wine yeasts, as they are usually diploid and homothallic (Mortimer 2000; Sipiczki 2011). Wine yeasts were historically submitted to RM, in order to improve the flavor profile of wine. For instance, Rous et al. (1983) and Giudici and Zinnato (1983) used RM to reduce higher alcohol production by *S. cerevisiae* wine strains.

Hybridization Yeasts, especially those belonging to *Saccharomyces* spp. and, more in general, to the winemaking species, feature the major evolutionary and technical advantages of sexual recombination. The sexual hybridization techniques, both intra- and interspecific ones, are the most efficient way to generate artificial diversity in yeasts. In the first case, the technique is termed "direct mating" and entails the highest degree of randomness and variability, given the putative high degree of heterozygosis in the wild genome (Fig. 12.5a). In the second case, also termed "mating of monosporic clones," the procedure starts from the constitution of homozygous lines from the wild progenitor (Fig. 12.5b). Then, mating is performed among the different obtained lines. Such a strategy allows a more thorough exploration of the phenotypic space, although suffering from an overall lower time efficiency (Solieri et al. 2015) compared to "direct mating," which is thus the most suitable for the rapid improvement of traits associated to quantitative trait loci (QTLs). In any case, genetic improvement programs based on monosporic cultures should comprise the extensive screening of desired phenotypic and genotypic strains in the progenitors (Verspohl et al. 2017).

In a recent study, Bonciani et al. (2016) applied a direct mating approach involving many wine strains of *S. cerevisiae*. Although the overall procedure was aimed at the obtainment of robust winemaking strains, some important considerations concerning GSH production were incidentally highlighted. In fact, the work showed that, generally speaking, GSH production seems to be subjected to the dominant effect of one of the parents or on the additive contribution of genes. In the first case, the produced titers were similar to those produced by one of the parents. In the second case, the produced levels of GSH were intermediate between those of the parental strains.

Metabolic Engineering The term "metabolic engineering" (ME) addresses the rational and targeted modification of both genetic and regulatory mechanisms in a given microorganism to optimize the production of specific metabolites or the expression of industrially relevant traits (Fig. 12.6; De Vero et al. 2017).

Due to the targeted nature of the modifications, ME strictly depends on the a priori knowledge of the molecular and regulatory network underlying either the considered traits or produced metabolites. Therefore, ME is easier to apply to model microorganisms, such as *S. cerevisiae* and *Escherichia coli*, or, more in general, to all those microorganisms with a greater amount of produced literature. *S. cerevisiae* is the most used yeast species in wine fermentations; being the model organism for eukaryotes, the species was the main subject of "omics" studies over the past century. Its genome was the first to be fully sequenced among the eukaryotes

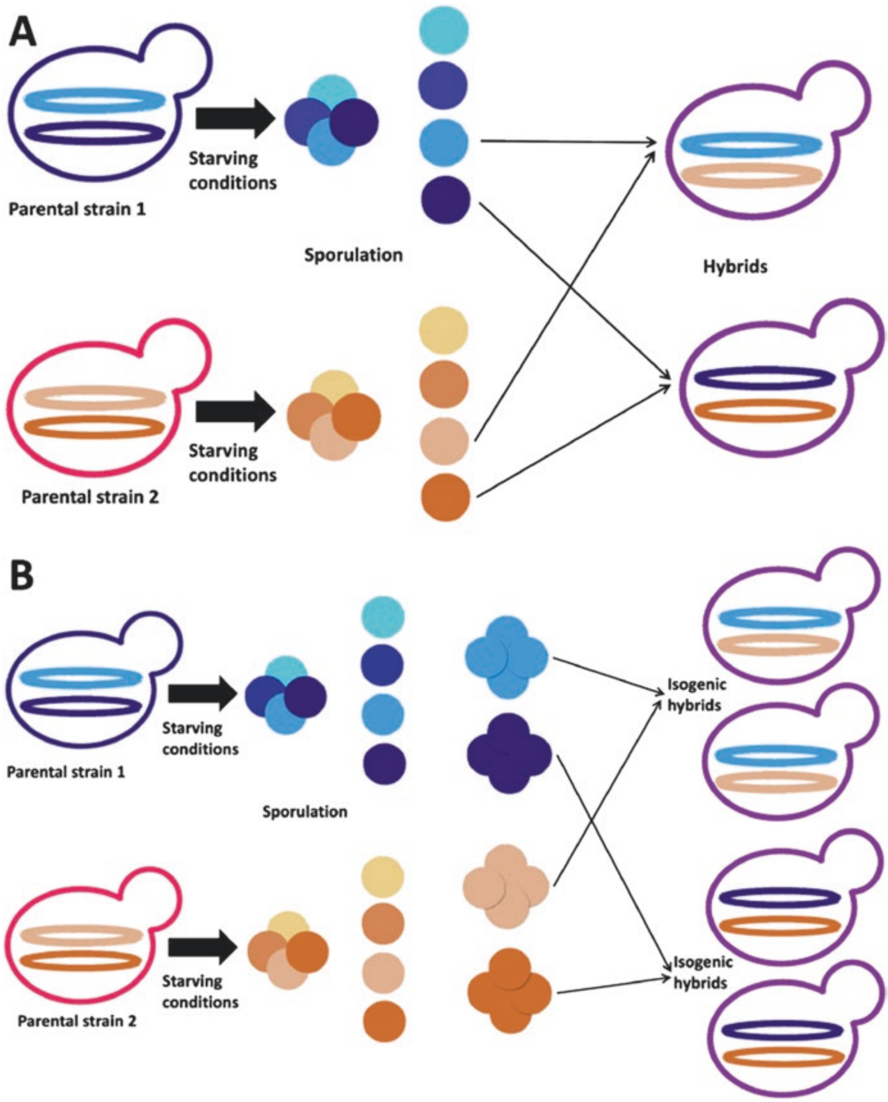


Fig. 12.5 Breeding strategies for intra- and interspecies hybrids, based on spore-to-spore mating. **a** Hybrids obtained by direct mating. **b** Isogenic hybrids obtained using monosporic clones (De Vero et al. 2017). Licensed under Creative Commons CC-BY 4.0

(Goffeau et al. 1996), which allowed the birth of several online databases over the years, encompassing genomic, proteomic, and metabolomic data.

However, this technique suffers some major drawbacks for its employment in the oenological industry and more in general in food industry. The first is related to the nature of oenologically relevant traits, which are nearly all determined by the concerted expression of QTLs spread throughout the genome, thus requiring the

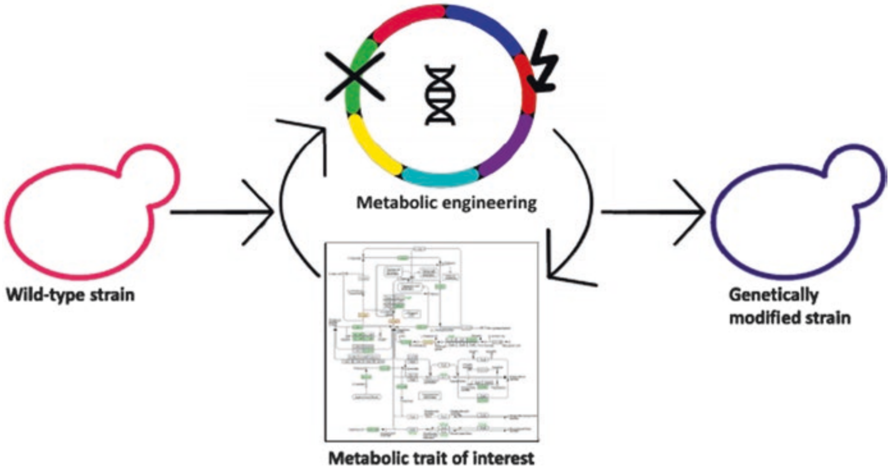


Fig. 12.6 Schematic overview of metabolic engineering strategies for genetic improvement of yeast strains (De Vero et al. 2017).

application of recursive strategies of targeted genetic modification. For instance, the trait of ethanol tolerance seems to be determined by as many as 250 QTLs spread in the genome (Pretorius 2000). Secondly, we should not neglect the high degree of genetic redundancy and pleiotropy inherent to the eukaryotic organisms: in such a context, strictly deterministic predictions are hardly feasible, even in the model *S. cerevisiae*. Finally, predictions become even more difficult when shifting from the well-treaded field of *S. cerevisiae* to other less conventional wine yeasts. Some authors reported ME strategies for improving GSH production in *S. cerevisiae* strains are required to survive in stressful environments like those occurring in the simultaneous saccharification and fermentation of lignocellulosic feedstocks (Ask et al. 2013; Qiu et al. 2015). They showed that the enhanced GSH content in *S. cerevisiae* strains had a relevant influence on their robustness.

Evolutionary Engineering The improvement of wine yeasts by evolutionary engineering, also referred to as "adaptive laboratory evolution" or "directed laboratory evolution," is a widely used approach. Evolutionary engineering is based on miming selection mechanisms active in nature. This is accomplished through the controlled application of a selective pressure on the microbial population, in order to select evolved strains with specific phenotypes (Sauer 2001; McBryde et al. 2006). The strength of the strategy is that no prior genetic knowledge is required to obtain new evolved strains.

Generally, the evolutionary engineering strategies consist of two basic steps: the strain randomization by mutation and/or recombination and the selection of evolved strains (De Vero et al. 2011). These strategies allow genetic drifts of the whole population, caused by mitotic or meiotic recombination events and/or accumulation

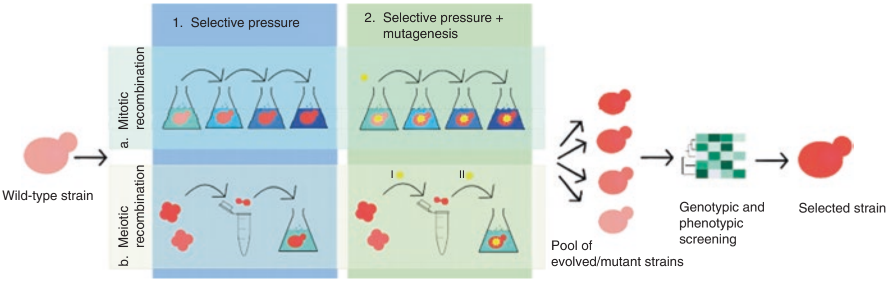


Fig. 12.7 Adaptive evolution strategies from the wild-type strain to the selected evolved strain (De Vero et al. 2017). Licensed under Creative Commons CC-BY 4.0

of natural or induced mutations. The mentioned mutations/recombinations are selected during cell growth, which favors the advantageous ones (Fig. 12.7).

Evolutionary engineering is particularly valuable to improve strains used in food and beverage technologies, where the application of genetically modified organisms (GMO) is prohibited or limited by legal restrictions. Therefore, since they have not undergone artificial genomic modifications, the use of yeast strains obtained by adaptive evolution approaches has a high degree of acceptance by the consumers, even in winemaking (De Vero et al. 2017).

In Fig. 12.8, a schematic model for GSH biosynthesis in *S. cerevisiae* is reported. The process involves two ATP-dependent steps: firstly, cysteine is linked with glutamate by γ-glutamylcysteine synthetase (encoded by *GSH1*) to form γ-glutamylcysteine. Secondly, glycine is added to this intermediate product by glutathione synthetase (encoded by *GSH2*) to form the final product (Li et al. 2004; Zechmann et al. 2011). GSH can chelate heavy metals by exploiting the cytosolic glutathione S-transferase enzyme, which enables the formation of a metal-GSH complex (Me (GSH) n). The Me (GSH) n complex is recognized as substrate by specific transporters (Ycf1p and Gex1p) leading to either vacuolar sequestration or export outside the cell (Ortiz et al. 1992; Duncan and Jamieson 1996; Mendoza-Cózatl et al. 2005).

Genetically Engineered Food Regulations

The authorization and labeling of genetically modified foods (GMFs) within the European Union are governed by the GMFs and Feed Regulation EC No. 1829/2003 (European Commission 2003a) and also by the Traceability and Labeling Regulation EC No. 1830/2003 (European Commission 2003b; Grujić and Blesić 2007). A GMF must not have adverse effects on human or animal health or the environment. The authorization process for GMFs is set out in Articles 5–7 of EC No. 1829/2003,

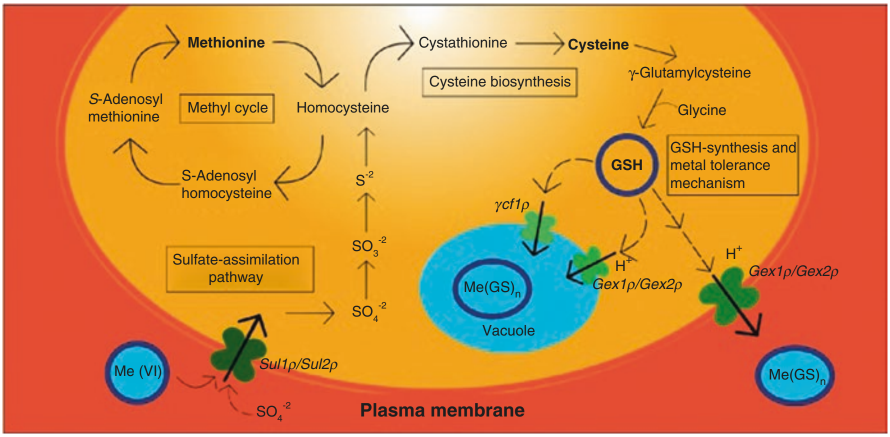


Fig. 12.8 Scheme representing the metabolism of sulfur, the synthesis of glutathione, and the GSH-mediated metal tolerance mechanism in *S. cerevisiae*. Me (VI), toxic metal oxyanion, sulfate analogue; Sul1p/Sul2p, sulfate transporters; Gex1p/Gex2p, yeast glutathione exchangers; Ycf1p, vacuolar glutathione S-conjugates pump; Me (GS) n, metal-GSH complex (De Vero et al. 2017). Licensed under Creative Commons CC-BY 4.0

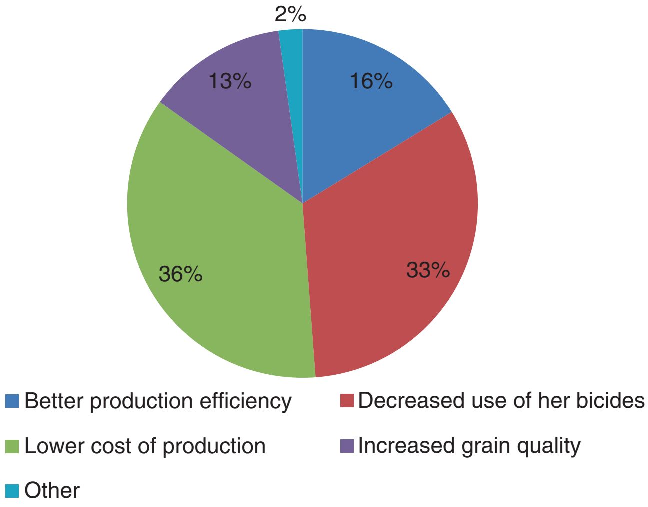
while information to assist in the preparation of the application dossier is detailed in Regulation EC No. 641/2004 (European Commission 2004; Giuseppe et al. 2010).

The GM labeling requires that a food or ingredient be labeled if it has a GM content of more than 0.9%. However, this does not mean that foods with a GM content of less than 0.9% are considered "GM-free." The terms "GM-free," "Non-GM," "Made with no GM ingredients," etc. have no legal definition. The voluntary labeling of food as "GM-free" or words to that effect is covered by the general food labeling legislation, and, as with all labels, its accuracy is the responsibility of the operator. A food that contains any level of GM ingredient should not bear a GM-free-type label as it is clearly misleading to the purchaser in breach of the general food labeling Directive (2000/13/EC) (European Commission 2000; Food Safety Authority of Ireland 'FSAI' 2005; Giuseppe et al. 2010).

In 2013, the new regulation is issued by the European Union (2013) that performs the traceability requirements for food containing or produced from GMOs, as set out in Regulation (EC) No. 1830/2003. This new regulation requires that accurate information provided by business operators is available to distributors and consumers to enable them to choose freely in an effective manner as well as to enable control and verification of labeling claims.

Both Canadian Food Inspection Agency (CFIA) and Health Canada are mandated to evaluate the nutritional value and safety of genetically modified foods (GMFs) released in Canada. Genetically modified (GM) or genetically engineered (GE) foods are foremost regulated by the Food and Drugs Act (R.S.C., 1985, c. F-27 2017) and its subordinate regulations (Consolidated Regulations of Canada 2017).

One of the big challenges facing the rapid development of these biotechnologies is the laws governing food. The US Department of Agriculture recently issued



Graph 12.1 Main advantages of the cultivation of GM crops in the opinion of Brazilian farmers (Panzarini et al. 2015). Licensed under Creative Commons CC-BY 4.0

(18 November 2015) temporary plans to revise existing regulations in the belief that these regulations are inappropriate to deal with many genetically modified foods (Zhang et al. 2016).

Genetically Modified Foods (GMFs) Still Questionable?!

This section is a face-to-face debate between two almost opposite positions regarding the application of genetic engineering in food production (Buiatti et al. 2013).

Through the qualitative research conducted by Panzarini and others in 2014 via a semi-structured questionnaire with 20 associated cooperatives of farmers at Campos Gerais region in Brazil, it was found that the views are linked to different experiences of each one with applications of GM crops. They mentioned that the benefits were set up as possible solutions to problems and difficulties in daily activities, in which this technology is seen as a tool to help growers improve the quality of their products. The production cost was highlighted among the main advantage of the cultivation of GM as shown in Graph 12.1 (Panzarini et al. 2015).

Modern biotechnology is helpful in enhancing taste, yield, shelf life, and nutritive values. This is also useful in food processing (fermentation and enzyme-involving processes). So, biotechnology is beneficial in erasing hunger, malnutrition, and diseases from developing and third-world countries. Modern biotechnology products are commercially reasonable; hence it can improve agriculture as well as food industry that will result in raise in income of poor farmers (Adenle 2011; Datta 2013; Rai and Shekhawat 2014). Following are some benefits of modern biotechnology applications in food processing.

Benefits

Enzymes

Enzymes are used in production and processing of food items specifically produced at the industrial level. From the second last decade of the twentieth century, food processing companies are using enzymes that are produced by genetically modified organisms (European Food Information Council "EUFIC" 2006). These enzymes comprise of proteases and carbohydrases. Genes for these enzymes have been cloned so as to get higher production in less time period. These enzymes are used for making cheese, curd, and flavoring food items. A major percentage of these enzymes is used in food industry as in the USA more than 50% of proteases and carbohydrases are used in food industry. These enzymes include rennin and α-amylase (Lawrence 1988).

The new food technologies or unsatisfactory performance of existed enzymes in established processes are considered as a strong pushing factor toward the producing of new enzymes via new technologies. The revolution of gene technology in the last two decades has enabled the enzyme manufacturers to produce enzymes satisfactory in terms of efficiency and quantity (Hatti-Kaul 2009; Hua et al. 2018).

The activity level of enzymes which are produced by organisms from a natural environment is often low and needs to be higher for industrial production. This increase is often achieved by mutations in the organisms or using recombinant organisms for enzyme production as the alternative strategy. Random and site-directed mutagenesis are becoming common genetic engineering practices to produce enzymes that own the stability properties prior to its production (Sahlin 1999;Buchholz et al. 2005;Hatti-Kaul 2009; Hua et al. 2018).

Applications in Food Industries In the food industries, make use of a variety of enzymes such as amylases and lipases. Amylases are used for liquefaction and saccharification of starch and also in the adjustment of flour, volume in baking, and bread softness (Kirk et al. 2002; Akoh et al. 2008; Turanli-Yildiz et al. 2012; Hua et al. 2018).

Regarding the other main group of enzymes used in food industries is constituted by lipases group, where it has many industrial advantages in food applications. For example, lipases play an important role as conditioning and stability factors of dough in the baking industry. Additionally, these work as in situ emulsifiers and contribute to the cheese flavor (Kirk et al. 2002). Hence, having safe lipases (non-toxic) is an important requirement of the food industry. This requirement applies to commercial lipase (isoform mixtures) prepared from *Candida rugosa*. The producing of pure lipase isoforms from *C. rugosa* is possible by computer modeling and protein engineering techniques (e.g., swapping, DNA shuffling) (Akoh et al. 2008;Ishige et al. 2005;Turanli-Yildiz et al. 2012; Hua et al. 2018).

Shelf Life

Fruits and vegetables are important components of the human diet. The post-harvest decay process of fruits and vegetables affects shelf life and limits transportation and storage resulting in post-harvest losses up to 50% of the total produce (Meli et al. 2010). Therefore, enhancement of fruit shelf life by slowing down of the post-harvest decaying process is among the targets of crop genetic improvement efforts.

Many juicy fruits possess short shelf life. For example, the tomato is used all over the world. In order to be shipped, tomatoes should be picked at the mature green stage. After picking, these are subjected to ethylene for ripening. Higher temperatures cause early ripening, while lower temperature destroys its taste. Meli et al. (2010) targeted the suppression of two N-glycan processing enzymes, α-mannosidase (α-Man), and *β*-D-N-acetylhexosaminidase (*β*-Hex) through RNAi approach in tomato, a climacteric fruit which requires ethylene to complete ripening process. Analysis of transgenic tomato revealed the enhanced fruit firmness and shelf life, due to the reduced rate of fruit softening. Similarly, RNAi-mediated suppression of α-Man and *β*-Hex in the non-climacteric fruit of capsicum delayed the fruit deterioration by ~7 days, and RNAi fruits of α-Man and *β*-Hex were ~2 times firmer than control (Ghosh et al. 2011).

Improving Food Nutrition

Every food item does not contain all essential components. That's why every food article is not possessing perfect nutrition. For example, rice is used as staple food in many countries of the world. But being devoid of vitamin A, it's not a perfect staple food. The use of biotechnological techniques has solved these problems through the introduction of foreign vitamin A gene (Sun 2008; Zhang et al. 2016).

Proteins and Essential Amino Acids More than half of worldwide protein production is attained from plants, but plant proteins lack some essential amino acids like lysine and sulfur-containing amino acids (Sun 2008). Corn is genetically modified, and it expresses proteins produced by soil bacteria *Bacillus thuringiensis* (Falk et al. 2002). To overcome the deficiency of essential amino acids, different biotechnological molecular processes are used and given below (Table 12.3).

Vitamins and Minerals These are compulsory food components; that's why, to avoid their deficiency, transgenic technology is used. Rice is one of the foods used as staple food in many countries of the world. But being deficient in vitamin A, rice is not a perfect staple food. Ye et al. (2000) developed nutritionally valuable "Golden rice" with *β*-carotene expression in the rice endosperm. The first provitamin-rich transgenic rice was produced by incorporating *crtI* gene and *psy* gene from bacteria and daffodils (Sun 2008). A variety of provitamin-rich rice can eliminate malnutrition and blindness from developing countries and third world (Falk et al. 2002). Similarly, super bananas with increased level of *β*-carotene were also developed by

Table 12.3 Crops modified with amino acid genes

Transgenic crop	Pathway of molecular modification	Amino acids enhanced	Foreign genes inserted
Potato	Manipulation of homologous protein	Most of amino acids	*AMA1*
Sunflower seed	Manipulation of gene expression	Sulfur-containing amino acid (methionine)	Gene encoding 2S albumin

Source: Haroon and Ghazanfar (2016). Licensed under Creative Commons CC-BY

transforming a phytoene synthase (PSY2a) gene from the Asupina banana variety (Mlalazi et al. 2012). Transformation of potato with a bacterial mini-pathway for β-carotene in a tuber-specific manner results in a "golden" potato (GP) tuber phenotype resulting from accumulation of provitamin A carotenoids (α- and β-carotene) and xanthophylls. Chitchumroonchokchai et al. (2017) suggest GP has the potential to contribute to the provitamin A and αTC nutritional requirements of populations at risk of vitamin A and E deficiency, especially in countries where potato is an important staple food. Their use for dietary interventions should, therefore, be considered by national and international programs aimed at preventing vitamin A and E deficiency.

Iron Iron is one of the most important minerals required for a healthy body. The countries which use rice as a staple food are more vulnerable to iron deficiency because rice is deficient in iron (WHO 2000). To resolve this problem, rice is transformed with a foreign gene encoding iron containing gene named ferritin. Transformed rice contains double content of rice as compared to non-transformed rice (Gura 1999).

Carbohydrates and Lipids Carbohydrates and lipids can be modified in transgenic plants. In the late twentieth century, amylopectin-rich potatoes and lauric acid-rich canola oil were produced through agricultural biotechnology (Sun 2008). Potatoes have been genetically modified by inserting a gene from bacteria that encodes enzyme involved in starch biosynthesis pathway. These GM potatoes contain 30–60% more starch (Falk et al. 2002).

Improve Yield

One of the positive aspects of genetic engineering is to provide food security through a variety of means, including increasing yield. For example, GM crops contribute 14% to increasing yield achieved by the USA, this contribution percentage equal an addition of more than 300 million acres of conventional crops (James 2013; Brookes and Barfoot 2014).

Bovine somatotropin is a hormone released by the pituitary gland. Previously this hormone was extracted from the brain of slaughtered calves but in low quantities. By insertion the gene encoding bovine Somatotropin in *Escherichia coli*, this hormone was obtained in high quantities. The somatotropin hormone results in 10–12% rise in milk production (Forge 1999; WHO 2000).

Enhancing Taste

Biotechnology has allowed scientists to produce fruits with better taste. GM foods with better taste include seedless watermelon, tomato, eggplant, pepper, cherries, etc. Elimination of seed from these food articles resulted in more soluble sugar content enhancing sweetness (Falk et al. 2002). Fermentation pathways are modified using biotechnology to add aroma in wine (Lawrence 1988).

Economic Benefits

Global economic gains from genetically modified foods were estimated at about $116 billion, which was achieved between 2006 and 2012, three times the rate achieved in the decade before 2006 (James 2013; Brookes and Barfoot 2014). The following economic details show how genetic engineering as a distinct technique contributed to these gains:

- Forty-two percent of these gains were achieved through the ability of genetically modified foods to resist pests and weeds.
- The remaining 58% resulted from reduced production costs (e.g., from reduced pesticide and herbicide usage).

Products for Therapeutic Purposes

One of the positive roles of genetic engineering is to enable edible plant parts to be a source of microbial antigens (Ellstrand and Hancock 1999; Hare and Chua 2002; Schafer et al. 2011). Thus, GE foods could serve as oral vaccines against different pathogens such as *Helicobacter pylori*, *Escherichia coli*, rabies virus, and hepatitis B (Ellstrand and Hancock 1999; Hare and Chua 2002;Reichman et al. 2006;Schafer et al. 2011;Aggarwal 2012; Nicolia et al. 2014).

Potential Risks and Concerns Regarding Genetic Engineering

For ecological and social advocates, the biggest problem in risk analysis of GMOs is that their effects cannot be predicted in its entirety. The human health risks include those unexpected, like allergies, toxicity, and intolerance. At the environment, the anticipated consequences are lateral or horizontal gene transfer, genetic pollution, and harmful effects on nontarget organisms (Nodari and Guerra 2003).

Panzarini et al. (2015) remarked from the interviews conducted with 20 GM producers in Brazil that the monopoly of seeds and inputs from companies was identified as the main disadvantage of the insertion of biotechnology in agriculture. On the other hand, GM producers pointed insurance transgenic and research that prove the risks to human health are few and delayed.

The debates and controversy on genetically modified foods have focused on main two axes (human health and environmental safety). Of course, there are many reasons that have led to increased consumer concerns about genetically modified foods, including (Baulcombe et al. 2014):

– The absence of the role that scientific communities are supposed to increase consumer awareness about safety and benefits achieved through genetic engineering techniques.
– Display foods genetically engineered in an inappropriate and unconvincing form for the consumer.
– Ethical aspects affect consumer decision so should be taken into consideration when introducing transgenic foods to consumers.
– The overall assessment of genetically modified foods is still incomplete, which negatively affects consumers.

Food Safety Concerns

Still, a question, which roams itself the head of many, is whether the GE food products pose any threat to human health. However, the ingestion of foreign DNA is an unlikely source of risk, as we ingest DNA daily as part of our regular diet of the plant-, animal-, and microbial-based products (Habibi-Najafi 2006). The potential food safety risks of genetic engineering can highlight in the following snapshots (Tietyen et al. 2000b):

– Unpredictable effects produced by transfer of genetic material
– Higher toxin levels than in traditional varieties
– Increased allergenic possibility
– The potential of transferring antibiotic resistance to organisms by marker genes

Environmental Concerns

Another potential risk is horizontal gene transfer. Transgenic organisms, when exposed to the natural environment, may transfer genes to other organisms resulting in spread of transgene everywhere. Consequences of this spread can destroy the ecosystem and other organisms. Horizontal transfer has been recorded in the lab (Haroon and Ghazanfar 2016). Hence the environmental risks included:

– Herbicide resistance: herbicide-tolerant GE crops have created weed resistance, causing pesticide use to increase by 70 million pounds between 1996 and 2003 (Benbrook 2012).
– Insect resistance: a report by Losey et al. (1999) raised fears about potential environmental damage to non-pest in`s. The pollen of Bt corn was suspected to adversely affect the monarch butterfly.
– Accelerated spread of antibiotic resistance due to the use of marker genes (Tietyen et al. 2000b).

Is It Possible to Trace Genetically Modified Foods (GMFs)?

Why Tracing GMFs Is Needed?

Responding to consumer demand increases for food traceability and also to soothe their concerns about genetically modified foods, Europe's regulators have extended traceability to GE food and feed.

In 2013, the European Commission released a regulation (EU, S.I. No. 268 of 2013) that enables tracing GMOs and GM food/feed products at all stages of the supply chain and makes labeling of these products possible. This regulation allows for close monitoring of potential effects on the human health and environment.

Traceability Approaches Currently Used

Traceability does not rely solely on documentation. Molecular biology means (polymerase chain reaction) even tiny traces of GMOs can be detected and identified provided that suitable testing tools exist for the GMO assumed to be present (Schreiber 1999; Mendoza et al. 2006). In the European Union, such a tool must be part of the application documents for the approval for commercialization.

Techniques Used for GM Animals as Case Study

At present, (real-time) PCR-based analysis is the method of choice for the routine analysis of food and feed samples for their GMO content (Bonfini et al. 2002; Holst-Jensen 2009; Zel et al. 2012). These methods are DNA based and are thus applicable for the detection of all (GM) organisms. They consist of targeting specific DNA sequences (between 60 and 200 base pairs long) for enzymatic amplification, revealing the presence (amplification) or absence (no amplification) of the target sequence. By following the amplification process fluorometrically (in real time), quantification of the initial amount of target sequence becomes possible (Lievens et al. 2015).

Detection and Quantification Targets The different types of DNA modifications can be evaluated for their capability to yield event-specific methods:

– Insertions: Insertions are the most straightforward group of modifications to detect. Insertion of a foreign sequence into the host genome inevitably generates two unique junctions. Even if the donor organism is the same species, the junctions at the insertion sites are wholly unique to the GMO, and specific detection is possible (Lievens et al. 2015). In addition, targeting genes or genetic elements inside the insertion may yield construct-specific and screening methods. The success of such screening strategies will largely depend on the origin of the inserted elements and whether its presence in the food chain may cause false-positive screening results (Lievens et al. 2015).
– Deletions: Deletions may not be detected by screening or construct-specific methods. However, similar to insertions, the removal of sequences usually creates a new, unique junction that may be targeted for amplification, thus rendering the event detectable. In addition, a deletion that is hard to detect is usually not interesting from the point of GM production because it may complicate screening for successful deletion in the early stages of the production process when the modification is attempted on a large number of cells (Lievens et al. 2015).
– Recombinase: Recombinase-mediated deletions form a more heterogeneous group of modifications. In research it is common to have two lines of mutants: one containing the recombinase gene and the other containing the deletion targeted genes. It is the offspring of these two lines which then form the functional mutants. It is thus possible to generate many different GM animals whose genomes all contain the same identical junctions flanking the recombinase gene. However, it is currently unclear if this practice will be applied in livestock (Lievens et al. 2015).
– Quantification: Quantification involves the parallel detection of two targets: a wild-type endogene and an event-specific transgene (both present in one copy per haploid genome). In addition, calibration curves for both genes are constructed using reference materials. The whole of results then allows accurate quantification of the GM content of the sample (Fig. 12.9) (Lievens et al. 2015). In current GM quantification strategies, the GM unique junction regions are used for the purpose of quantification. And as explained in the above, similar targets are expected to be present in GM animals.

Species Markers A crucial point in the current identification strategies, using real-time PCR, is the requirement for highly specific endogene markers that show no cross-reactivity with other species and are able to detect all (commercial) varieties and/or subspecies of the given organism. On this basis, many PCR-based detection and identification strategies for animal ingredients have already been published (e.g., see Table 12.4), and the identification of (non-GMO) animal ingredients in processed foods by means of PCR has been gaining importance, most notably in the field of food authentication (Lievens et al. 2015).

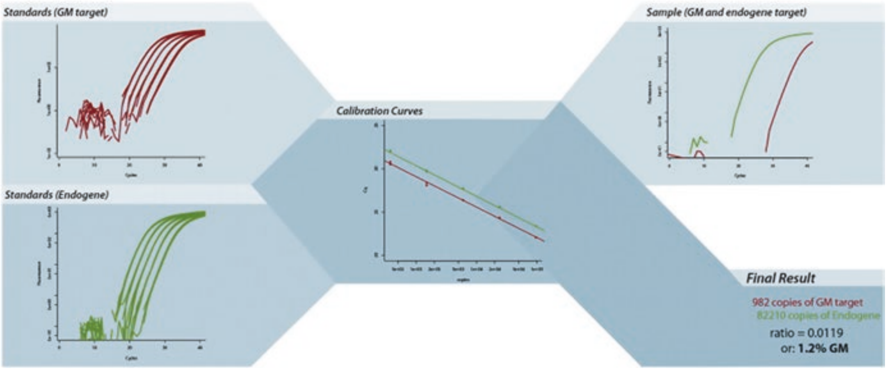


Fig. 12.9 GM quantification strategy. Real-time PCR detection is carried out for two targets: a wild-type endogene and an event-specific transgene. Both reactions are performed on dilution series of a reference material (calibration curves) and on the actual sample. The calibration curves then allow the calculation of the GM content of the sample. (Source: Lievens et al. 2015. Reproduced with permission of Elsevier)

Table 12.4 Examples of animal species-specific PCR markers used in food authentication

Species	Target origin	Gene	Amplicon size (bp)
Cow	Mitochondrial	D-loop	513
	Mitochondrial	12S	252
	Mitochondrial	Cytochrome b	120
Goat	Mitochondrial	12S	117
Chicken	Mitochondrial	D-loop	256
	Mitochondrial	ND5/cytochrome b	117
	Mitochondrial	Cytochrome b	106
Tuna	Mitochondrial	16S	63

Source: Adapted from Lievens et al. (2015). (Reproduced with permission of Elsevier)

GM Animal Product Derivatives Next to samples that are either pure meat or have meat as their main ingredient, there are a large number of processed food products that contain animal derivatives. Although the DNA content of food has been of increasing interest to control authorities worldwide (El Sheikha et al. 2017), data that show which food or food fractions still contain DNA are not readily available. As a consequence, the degree to which a single animal derivative ingredient is detectable by DNA-based methods in a sample (if at all) is currently not known and is likely to differ between products and ingredients. In such cases, the detection of GM animal content and its quantification may be more difficult and/or require specific DNA extraction methods or entirely different approaches (Lievens et al. 2015).

Conclusion

Genetic engineering technology is one of the advanced technologies that has potential to solve problems of malnutrition, hunger, and poverty through the increasing of production and enhanced quality and safety of food. Biotechnology has the potency to solve many health- and nutrition-related problems of people of developing countries and third world. Organizations like WHO, FDA, etc. should cooperate with governments to make biosafety laws and commercialization of GMFs. One of the weak areas in the field of food biotechnology is labeling. Proper and positive labeling is required for successful commercialization of GMFs. Another weak area is a lack of research. Research should be done to prove or falsify the claims against biotechnology. In spite of a lot of advancements, still a large number of people oppose genetically modified foods (GMFs), raising fears about potential safety and environmental risks. Debates and seminars should be conducted to raise the trust and confidence of people about GMFs. From this perspective, the importance of traceability arises as one of the proposed solutions to address the challenges facing genetically modified foods, especially for consumers, which will be reflected positively on the improvement of the marketing situation and increased economic benefits of genetically modified foods.

References

Adenle AA (2011) Response to issues on GM agriculture in Africa: are transgenic crops safe. BMC Res Notes 4:1–6

Aggarwal S (2012) What's fueling the biotech engine – 2011 to 2012. Nat Biotechnol 30(12):1191–1197

Akoh CC, Chang SW, Lee GC, Shaw JF (2008) Biocatalysis for the production of industrial products and functional foods from rice and other agricultural produce. J Agric Food Chem 56(22):10445–10451

Ask M, Mapelli V, Höck H, Olsson L, Bettiga M (2013) Engineering glutathione biosynthesis of *Saccharomyces cerevisiae* increases robustness to inhibitors in pretreated lignocellulosic materials. Microb Cell Factories 12:87

Barre P, Vezinhet F, Dequin S, Blondin B (1993) Genetic improvement of wine yeasts. In: Fleet GH (ed) Wine microbiology and biotechnology. Harwood Academic Publishers, Chur

Baulcombe DD, Jones J, Pickett J, Puigdomenech JP (2014) GM science update: a report to the council for science and technology. March 2013. http://centaur.reading.ac.uk/36228/1/GM%20Science%20Update%20-%20Report%20to%20CST%20110314.pdf. Accessed 20 Feb 2018

Belhaj K, Chaparro-Garcia A, Kamoun S, Patron NJ, Nekrasov V (2015) Editing plant genomes with CRISPR/Cas9. Curr Opin Biotechnol 32:76–78

Benbrook CM (2012) Impacts of genetically engineered crops on pesticide use in the U.S. – the first sixteen years. Environ Sci Eur 24:24. https://link.springer.com/article/10.1186/2190-4715-24-24#Bib1. Accessed 20 Feb 2018

Bonciani T, Solieri L, De Vero L, Giudici P (2016) Improved wine yeasts by direct mating and selection under stressful fermentative conditions. Eur Food Res Technol 242(6):899–910

Bonfini L, Kay S, Heinze P, Van den Eede G (2002) Report on GMO detection identification and quantification methods submitted to collaborative studies. European Communities, EUR 20383 EN:1–29

Brookes G, Barfoot P (2014) Economic impact of GM crops: the global income and production effects 1996–2012. GM Crops Food 5(1):65–75

Buchholz K, Kasche K, Bornscheuer UT (2005) Biocatalysts and enzyme technology. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Buiatti M, Christou P, Pastore G (2013) The application of GMOs in agriculture and in food production for a better nutrition: two different scientific points of view. Genes Nutr 8:255–270

Chitchumroonchokchai C, Diretto G, Parisi B, Giuliano G, Failla ML (2017) Potential of golden potatoes to improve vitamin A and vitamin E status in developing countries. PLoS One 12(11):e0187102

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F (2013) Multiplex genome engineering using CRISPR/Cas systems. Science 339(6121):819–823

Consolidated Regulations of Canada (CRC) (2017) Food and drug regulations. Food and Drug Act, C.R.C., c. 870, Regulations are current to October 25, 2017 and last amended on June 20, 2017. http://laws-lois.justice.gc.ca/PDF/C.R.C.,_c._870.pdf. Accessed 20 Feb 2018

Datta A (2013) Genetic engineering for improving quality and productivity of crops. Agric Food Secur 2:15. http://www.agricultureandfoodsecurity.com/content/2/1/15. Accessed 20 Feb 2018

De Vero L, Solieri L, Giudici P (2011) Evolution-based strategy to generate non-genetically modified organisms Saccharomyces cerevisiae strains impaired in sulfate assimilation pathway. Lett Appl Microbiol 53:572–575

De Vero L, Bonciani T, Verspohl A, Mezzetti F, Giudici P (2017) High-glutathione producing yeasts obtained by genetic improvement strategies: a focus on adaptive evolution approaches for novel wine strains. AIMS Microbiol 3(2):155–170

DeMayo FJ, Spencer TE (2014) CRISPR bacon: a sizzling technique to generate genetically engineered pigs. Biol Reprod 91(3):79

Dequin S (2001) The potential of genetic engineering for improving brewing, wine-making and baking yeasts. Appl Microbiol Biotechnol 56:577–588

Diehl P (2017) What are GMOs and how are they made? https://www.thebalance.com/what-are-gmos-and-how-are-they-made-375620. Accessed 20 Feb 2018

Duncan WS, Jamieson DJ (1996) Glutathione is an important antioxidant molecule in the yeast *Saccharomyces cerevisiae*. FEMS Microbiol Lett 141:207–212

El Sheikha AF, Mokhtar NFK, Amie C, Lamasudin DU, Isa NM, Mustafa S (2017) Authentication technologies using DNA-based approaches for meats and halal meats determination. Food Biotechnol 31(4):281–315

El Sheikha AF (2018a) How to determine the geographical origin of food by molecular techniques? In: El Sheikha AF, Levin RE, Xu J (eds) Molecular techniques in food biology: safety, biotechnology, authenticity & traceability. Wiley, Oxford

El Sheikha AF (2018b) Revolution in fermented foods: from artisan household technology to era of biotechnology. In: El Sheikha AF, Levin RE, Xu J (eds) Molecular techniques in food biology: safety, biotechnology, authenticity & traceability. Wiley, Oxford

Ellstrand NPH, Hancock JF (1999) Gene flow and introgression from domesticated plants into their wild relatives. Annu Rev Ecol Syst 30:539–563

European Commission (2000) Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs. 6.5.2000. Official Journal of the European Communities: L 109/29–42. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:109:0029:0042:EN:PDF. Accessed 20 Feb 2018

European Commission (2003a) Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. 18.10.2003. Official

Journal of the European Union: L 268/1–23. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:268:0001:0023:EN:PDF. Accessed 20 Feb 2018

European Commission (2003b) Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. 18.10.2003. Official Journal of the European Union: L 268/24–28. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:268:0024:0028:EN:PDF. Accessed 20 Feb 2018

European Commission (2004) Commission Regulation (EC) No 641/2004 of 6 April 2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the application for the authorisation of new genetically modified food and feed, the notification of existing products and adventitious or technically unavoidable presence of genetically modified material which has benefited from a favourable risk evaluation.7.4.2004. Official Journal of the European Union: L 102/14–25. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:102:0014:0025:EN:PDF. Accessed 20 Feb 2018

European Food Information Council (EUFIC) (2006) Modern biotechnology in food: applications of food biotechnology: enzymes, September 6, 2006. http://www.eufic.org/en/food-production/article/modern-biotechnology-in-food-applications-of-food-biotechnology-enzymes. Accessed 20 Feb 2018

European Union Regulations (2013) Genetically Modified Foodstuffs Regulations 2013, Statutory Instruments (S.I. No. 268 of 2013). https://www.fsai.ie/uploadedFiles/Legislation/Food_Legisation_Links/Genetically_Modified_Organisms_(GMOs)/SI268_2013.pdf. Accessed 20 Feb 2018

Falk MC, Chassy BM, Harlander SK, Hoban TJ IV, McGloughlin MN, Akhlaghi AR (2002) Food biotechnology: benefits and concerns. J Nutr 132:1384–1390

FAO (Food and Agricultural Organization of the United Nations) (2009) Feeding the world, eradicating hunger. World summit on food security. Food and Agricultural Organization of the United Nations, Rome. WSFS 2009/INF/2

Farmer BH (1986) Perspectives on the ‘Green Revolution’ in South Asia. Mod Asian Stud 20(1):175–199

FDA (2015) Genetically engineered animals: consumer Q&A, August 12, 2015. https://www.fda.gov/animalveterinary/developmentapprovalprocess/geneticengineering/geneticallyengineeredanimals/ucm113672.htm. Accessed 20 Feb 2018

FDA (2017) Foods derived from plants produced using genome editing. April 12, 2017. https://www.fda.gov/Food/IngredientsPackagingLabeling/GEPlants/ucm537109.htm. Accessed 20 Feb 2018

Food Safety Authority of Ireland (FSAI) (2005) GM food survey 2004, Food labelled with “GM free” type declarations. https://www.fsai.ie/uploadedFiles/Monitoring_and_Enforcement/Monitoring/Surveillance/GM_survey_2004.pdf. Accessed 20 Feb 2018

Forge F (1999) Recombinant bovine somatotropin (*rbST*). ParliamentaryResearch Branch, Canada

Ghosh S, Meli VS, Kumar A, Thakur A, Chakraborty N, Chakraborty S, Datta A (2011) The N-glycan processing enzymes alpha-mannosidase and beta-D-N-acetylhexosaminidase are involved in ripening-associated softening in the non-climacteric fruits of capsicum. J Exp Bot 62(2):571–582

Giudici P, Zinnato A (1983) Influenza dell’uso di mutanti nutrizionali sulla produzione di alcooli superiori. Vignevini 10:63–65. [In Italian]

Giuseppe E, Monica S, GianFranco G (2010) Science for food safety, security and quality: a review – part 1. Qual Life 1(1):26–40

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327(5967):812–818

Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG (1996) Life with 6000 genes. Science 274(5287):546–567

GRACE Communications Foundation (2017) Genetic engineering, Food Program. http://www.sustainabletable.org/264/genetic-engineering. Accessed 20 Feb 2018

Grujić S, Blesić M (2007) Food regulations. Faculty of Technology, Banja Luka

Gura T (1999) New genes boost rice nutrients. Science 285:994–995

Habibi-Najafi MB (2006) Food biotechnology and its impact on our food supply. Glob J Biotechnol Biochem 1(1):22–27

Hare PD, Chua NH (2002) Excision of selectable marker genes from transgenic plants. Nat Biotechnol 20(6):575–580

Haroon F, Ghazanfar M (2016) Applications of food biotechnology. J Ecosyst Ecography 6(4):215. https://www.omicsonline.org/open-access/applications-of-food-biotechnology-2157-7625-1000215.php?aid=81688. Accessed 20 Feb 2018

Hatti-Kaul R (2009) Enzyme production. Biotechnology, Vol. V. Encyclopedia of Life Support Systems (EOLSS). p 1–7. www.eolss.net/EolsssampleAllChapter.aspx. Accessed 02 Feb 2015

Hesham EA (2010) Genetic improvement of yeast for bioethanol fermentation, Presented to Genetics Department, Faculty of Agriculture Assiut University. www.isosugar.org/Egypt/TL.2.2.pdf. Accessed 02 Feb 2015

Holst-Jensen A (2009) Testing for genetically modified organisms (GMOs): past, present and future perspectives. Biotechnol Adv 27(6):1071–1082

Hsieh Y-HP, Ofori JA (2007) Innovations in food technology for health. Asia Pac J Clin Nutr 16(Suppl 1):65–73

Hua W, El Sheikha AF, Xu J (2018) Molecular techniques for making recombinant enzymes used in food processing. In: El Sheikha AF, Levin RE, Xu J (eds) Molecular techniques in food biology: safety, biotechnology, authenticity & traceability. Wiley, Oxford

Institute of Medicine and National Research Council (2004) Safety of genetically engineered foods: approaches to assessing unintended health effects. The National Academies Press, Washington, DC. https://doi.org/10.17226/10977. Accessed 20 Feb 2018

International Food Information Council Foundation (IFIC) (2013) Food biotechnology: a communicator's guide to improving understanding. 3rd edn. April 16, 2013. http://www.foodinsight.org/education/food-biotechnology-communicators-guide-improving-understanding. Accessed 20 Feb 2018

International Food Information Council Foundation (IFIC) (2014) IFIC 2014 food technology survey: consumers support food biotechnology's use for certain benefits. July 10, 2014. http://www.foodinsight.org/newsletters/ific-2014-food-technology-survey-consumers-support-food-biotechnology%E2%80%99s-use-certain. Accessed 20 Feb 2018

International Food Information Council Foundation (IFIC) (2015) Food & health survey 2015, the 2015 food & health survey was conducted by Greenwald & Associates of Washington D.C., March 13–26, 2015. http://www.foodinsight.org/sites/default/files/2015%20Food%20And%20Health%20Survey-%20Executive%20Summary%20-%20Final.pdf. Accessed 20 Feb 2018

International Service for the Acquisition of Agri-biotech Applications (ISAAA) (2016) Global status of commercialized biotech/GM crops: 2016, ISAAA Brief No. 52. ISAAA, Ithaca. http://www.isaaa.org/resources/publications/briefs/52/executivesummary/default.asp. Accessed 20 Feb 2018

Irfan M, Datta A (2017) Improving food nutritional quality and productivity through genetic engineering. Int J Cell Sci Mol Biol 2(1):555576. http://59.163.192.83:8080/jspui/handle/123456789/736. Accessed 20 Feb 2018

Ishige T, Honda K, Shimizu S (2005) Whole organism biocatalysis. Curr Opin Chem Biol 9:174–180

James C (2013) Global status of commercialized biotech/GM crops: 2013, ISAAA Brief No. 46, 2013

Jha A (2011) GM chickens created that could prevent the spread of bird flu, the guardian. January 13, 2011. https://www.theguardian.com/science/2011/jan/13/gm-chickens-bird-flu-influenza. Accessed 20 Feb 2018
Kirk O, Borchert TV, Fuglsang CC (2002) Industrial enzyme applications. Curr Opin Biotechnol 13(4):345–351
Lawrence R (1988) New applications of biotechnology in the food industry. Biotechnology and the food supply: proceedings of symposium
Li Y, Wei G, Chen J (2004) Glutathione: a review on biotechnological production. Appl Microbiol Biotechnol 66:233–242
Li S, Yang X, Yang S, Zhu M, Wang X (2012) Technology prospecting on enzyme: application, marketing, and engineering. Comput Struct Biotechnol J 2(3):e201209017
Lievens A, Petrillo M, Querci M, Patak A (2015) Genetically modified animals: options and issues for traceability and enforcement. Trends Food Sci Technol 44(2):159–176
Losey JE, Rayor LS, Carter ME (1999) Transgenic pollen harms monarch larvae. Nature 399:214
McBryde C, Gardner JM, De Barros LM, Jiranek V (2006) Generation of novel wine yeast strains by adaptive evolution. Am J Enol Vitic 57(4):423–430
Meli VS, Ghosh S, Prabha TN, Chakraborty N, Chakraborty S, Datta A (2010) Enhancement of fruit shelf life by suppressing N-glycan processing enzymes. Proc Natl Acad Sci U S A 107(6):2413–2418
Mendoza A, Fernández S, Cruz MA, Rodríguez-Perez MA, Resendez-Perez D, Barrera Saldaña HA (2006) Detection of genetically modified maize food products by the polymerase chain reaction. Cienc Tecnol Aliment 5(3):175–181
Mendoza-Cózatl D, Loza-Tavera H, Hernández-Navarro A, Moreno-Sánchez R (2005) Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants. FEMS Microbiol Rev 29:653–671
Mlalazi B, Welsch R, Namanya P, Khanna H, Geijskes RJ, Harrison MD, Harding R, Dale JL, Bateson M (2012) Isolation and functional characterization of banana phytoene synthase genes as potential cisgenes. Planta 236(5):1585–1598
Mortimer RK (2000) Evolution and variation of the yeast (*Saccharomyces*) genome. Genome Res 10:403–409
Nicolia A, Manzo A, Veronesi F, Rosellini D (2014) An overview of the last 10years of genetically engineered crop safety research. Crit Rev Biotechnol 34(1):77–88
Nodari RO, Guerra MP (2003) Plantas transgênicas e seus produtos: impactos, riscos e segurança alimentar. Rev Nutr 16:105–116
Ortiz DF, Kreppel L, Speiser DM, Scheel G, McDonald G, Ow DW (1992) Heavy metal tolerance in the fission yeast requires an ATP-binding cassette-type vacuolar membrane transporter. EMBO J 11:3491–3499
Panzarini NH, Matos EASDA, Wosiack PA, Bittencourt JVM (2015) Biotechnology in agriculture: the perception of farmers on the inclusion of Genetically Modified Organisms (GMOs) in agricultural production. Afr J Agric Res 10(7):631–636
Park A (2015) 7 things you need to know about GMO salmon, time. November 19, 2015. http://time.com/4120648/fda-approved-aquabounty-gmo-salmon/. Accessed 20 Feb 2018
Patel R (2013) The long green revolution. J Peasant Stud 40(1):1–63
Perez-Gago MB, Serra M, del Rıo MA (2006) Color change of fresh-cut apples coated with whey protein concentrate-based edible coatings. Postharvest Biol Technol 39:84–92
Pretorius IS (2000) Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast 16:675–729
Qiu Z, Deng Z, Tan H, Zhou S, Cao L (2015) Engineering the robustness of *Saccharomyces cerevisiae* by introducing bifunctional glutathione synthase gene. J Ind Microbiol Biotechnol 42:537–542
R.S.C., 1985, c. F-27 (2017) An act respecting food, drugs, cosmetics and therapeutic devices, Published by the Minister of Justice, Last amended on June 22, 2017. http://laws-lois.justice.gc.ca/PDF/F-27.pdf. Accessed 20 Feb 2018

Rai MK, Shekhawat NS (2014) Recent advances in genetic engineering for improvement of fruit crops. Plant Cell Tissue Organ Cult 116(1):1–15
Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F (2013) Genome engineering using the CRISPR-Cas9 system. Nat Protoc 8(11):2281–2308
Reichman JR, Watrud LS, Lee EH, Burdick CA, Bollman MA, Storm MJ, King GA, Mallory-Smith C (2006) Establishment of transgenic herbicide-resistant creeping bentgrass (*Agrostis stolonifera* L.) in nonagronomic habitats. Mol Ecol 15(13):4243–4255
Richt JA, Kasinathan P, Hamir AN, Castilla J, Sathiyaseelan T, Vargas F, Sathiyaseelan J, Wu H, Matsushita H, Koster J, Kato S, Ishida I, Soto C, Robl JM, Kuroiwa Y (2007) Production of cattle lacking prion protein. Nat Biotechnol 25(1):132. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2813193/. Accessed 20 Feb 2018
Rous CV, Snow R, Kunkee RE (1983) Reduction of higher alcohols by fermentation with a leucine-auxotrophic mutant of wine yeast. J Inst Brew 89:274–278
Sahlin P (1999) Fermentation as a method of food processing (production of organic acids, pH-development and microbial growth in fermenting cereals) Licentiate thesis May, Lund University
Sauer U (2001) Evolutionary engineering of industrially important microbial phenotypes. In: Nielsen J, Eggeling L, Dynesen J, Gárdonyi M, Gill RT, de Graaf AA, Hahn-Hägerdal B, Jönsson LJ, Khosla C, Licari R, McDaniel R, McIntyre M, Miiller C, Nielsen J, Cordero Otero RR, Sahm H, Sauer U, Stafford DE, Stephanopoulos G, Wahlbom CE, Yanagimachi KS, van Zyl WH (eds) Metabolic engineering. Springer, Berlin
Schafer MG, Ross AA, Londo JP, Burdick CA, Lee EH, Travers SE, Van de Water PK, Sagers CL (2011) The establishment of genetically engineered canola populations in the US. PLoS One 6(10):e25736
Schreiber GA (1999) Challenges for methods to detect genetically modified DNA in foods. Food Control 10:351–352
Secretariat of the Convention on Biological Diversity (2000) Cartagena protocol on biosafety to the convention on biological diversity: text and annexes. Secretariat of the Convention on Biological Diversity, Montreal. https://www.cbd.int/doc/legal/cartagena-protocol-en.pdf. Accessed 20 Feb 2018
Sipiczki M (2011) Diversity, variability and fast adaptive evolution of the wine yeast (*Saccharomyces cerevisiae*) genome—a review. Ann Microbiol 61:85–93
Slack JMW (2014) Genes: a very short introduction. Oxford University Press, New York
Solieri L, Verspohl A, Bonciani T, Caggia C, Giudici P (2015) Fast method for identifying inter- and intraspecies *Saccharomyces* hybrids in extensive genetic improvement programs based on yeast breeding. J Appl Microbiol 119:149–161
Sun SS (2008) Applications of agricultural biotechnology to improve food nutrition and health care products. Asia Pac J Clin Nutr 17:87–90
Tietyen JL, Garrison ME, Bessin RT Hildebrand DF (2000a) Food biotechnology, educational programs of the Kentucky Cooperative Extension Service. http://www2.ca.uky.edu/agcomm/pubs/brei/brei3/brei3.htm. Accessed 20 Feb 2018
Tietyen JL, Garrison ME, Bessin RT Hildebrand DF (2000b) Food biotechnology teaching guide, educational programs of the Kentucky Cooperative Extension Service. http://www2.ca.uky.edu/agcomm/pubs/brei/brei3tg/brei3tg.htm. Accessed 20 Feb 2018
Ting J, Xu R, Xu J (2018) Molecular identification and distribution of yeasts in fruits. In: El Sheikha AF, Levin RE, Xu J (eds) Molecular techniques in food biology: safety, biotechnology, authenticity & traceability. Wiley, Oxford
Turanli-Yildiz B, Alkim C, Cakar ZP (2012) Protein engineering methods and applications. In: Kaumaya P (ed) Protein engineering, InTech, Rijeka. http://cdn.intechopen.com/pdfs-wm/29172.pdf. Accessed 20 Feb 2018
Verspohl A, Solieri L, Giudici P (2017) Exploration of genetic and phenotypic diversity within Saccharomyces uvarum for driving strain improvement in winemaking. Appl Microbiol Biotechnol 101:2507–2521

Visk D (2017) CRISPR applications in plants, a report from the plant and animal genomics conference. Genetic Engineering & Biotechnology News (GEN), February 14, 2017. https://www.genengnews.com/gen-exclusives/crispr-applications-in-plants/77900846. Accessed 20 Feb 2018

WHO (2014) Frequently asked questions on genetically modified foods, food safety. http://www.who.int/foodsafety/areas_work/food-technology/faq-genetically-modified-food/en/. Accessed 20 Feb 2018

WHO (2017) Food, genetically modified, health topics. http://www.who.int/topics/food_genetically_modified/en/. Accessed 20 Feb 2018

WHO (World Health Organization) (2000) Nutrition for health and development: a global agenda for combating malnutrition. World Health Organization, Geneva

Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287(5451):303–305

Zechmann B, Liou LC, Koffler BE, Horvat L, Tomašić A, Fulgosi H, Zhang Z (2011) Subcellular distribution of glutathione and its dynamic changes under oxidative stress in the yeast *Saccharomyces cerevisiae*. FEMS Yeast Res 11:631–642

Zel J, Milavec M, Morisset D, Plan D, Van den Eede G, Gruden K (2012) How to reliably test for GMOs. Springer, London

Zhang C, Wohlhueter R, Zhang H (2016) Genetically modified foods: a critical review of their promise and problems. Food Sci Human Wellness 5:116–123

Zhao Y, McDaniel M (2005) Sensory quality of foods associated with edible film and coating systems and shelf-life extension. In: Han JH (ed) Innovations in food packaging. Elsevier Academic Press, San Diego

Chapter 13
Innovative and Safe Packaging Technologies for Food and Beverages: Updated Review

Ishrat Majid, Mamta Thakur, and Vikas Nanda

Abstract The diverse consumer demand is the main drive for innovations in food packaging. Active as well as intelligent packaging is undoubtedly a huge milestone of the packaging sector in this era extending the shelf life as well as maintaining the food quality. Bioactive packaging, a new approach, has a great role in improving the consumer's health. Nanotechnology like a magical spell has revolutionized the packaging from lighter, more robust, and flexible films to the smart packaging monitoring the food condition. Nanoscale innovations are bringing the packaging area to a brand new unimaginable distinction. The emerging packaging technologies have a monumental influence on several facets of the food segment by minimizing the food wastage, spoilage, food-borne diseases' breakthrough, recalls, and retailer and consumer complaints. This chapter deals with the novel packaging technologies that lower the pathogen detection time, improve the food safety, and control the food packaging and quality all over the supply chain.

Keywords Active packaging · Intelligent packaging · Bioactive packaging · Nanotechnology · Responsive packaging · Microwavable packaging · Edible packaging

Introduction

Food and beverage without a package are unimaginable. Packaging is therefore a necessity for maintaining the quality, safety, and integrity of produce as well as processed products from the farm or plant to the end use. The food and beverage packaging industry has covered a long distance from where it began with the basic carton packaging which was performed with the motive of containment and

I. Majid (✉) · M. Thakur · V. Nanda
Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology, Longowal, Punjab, India

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_13

transportation only. Traditionally, the major objective of the packaging is food containment; its protection; communication of information like nutritional content, how to use, manufacturer's contact details, etc.; and consumer convenience. However, innovative and safe packaging is the need of time due to the rapid rising consumers' demands towards the fresher, safer, less processed, highly convenient, and prolonged shelf life foods. The current drift of changing lifestyle, eating habits, relentless and extensive competition in market, retail practices, logistics efficiency, and sustainability is the basic driving force behind the requirement of innovative packaging without harming the safety and quality characteristics of food (Dainelli et al. 2008). Further, the food and beverage safety is the main priority given by the current food legislation. The issues of food-borne outbreaks from the past demand the advanced packaging methods to ensure the safety of food.

The recent advances in packaging include active packaging (such as oxygen/CO_2/ethylene scavengers, moisture control agents, etc.), intelligent packaging, antimicrobial packaging, nano-packaging, aseptic packaging, packaging novel mechanisms that regulate the volatile flavours and aromas, advancements in food distribution technology (like electronic product codes and radio-frequency identification), and many more. Active packaging, one of the best examples of innovative packaging, includes basic primary functions of packaging and makes it possible to read, see, feel, or smell the packaged food characteristics. Smart packaging, which is considered as an advanced active packaging, involves the use of sensors. Intelligent packaging which contains indicators/sensors provides us valuable information about the status of food or its surrounding medium (Kerry 2014). It simply accompanies the communication function of conventional packaging and informs the customers about internal or external changes in the product's surrounding.

Antimicrobial packaging that plays a significant part to increase the food and beverage life on shelf is on demand these days due to the microbial contamination leading to health hazard outbreaks. Nanotechnology is offering new opportunities to improve the barrier and mechanical properties of conventional materials and to develop the sensors and novel packaging designs. The use of nanoparticles containing composite materials is considered to be the success of food packaging to control the microbial growth resulting in spoilage. The nanomaterials containing nanoparticles of zinc oxide (ZnO), silver (Ag), and titanium dioxide (TiO_2) have been used recently in the packaging systems. Further, research has been carrying out to explore the potential and safety of use of organic nanomaterials like chitosan and antimicrobial peptides in food packaging. The latest tracking systems are capable of tracking the food packages from the field to fork. A universal product code is being embossed onto the packages for facilitating the checkout as well as distribution control. Recent emerging innovations include sensing the surface variations through the palms and fingertips, any message either sound or verbal, and the odour released under active packaging gamut (Landau 2007).

Active Packaging

Active packaging is typically a supplement of the traditional preservation/protection function of a package conferring the several safety benefits to food products because of the current developments in packaging, material science, and complex consumer desires. Active packaging is generally defined as "addition of definite compounds to the package entity which absorb or release the substances from or into the packaged products or the environment to maintain the nutritional as well as sensory quality while increasing the shelf life and securing the microbial safety of products" (Camo et al. 2008). Active packaging is termed so because package here it is active that it interacts with the food, package, and headspace of the package to maintain the food quality to optimum. The decency of active packaging relies on the inherent characteristics of polymer and incorporation of particular components to the packaging materials (Gontard 2000). It is such that a packaging system offers surplus function besides the protection. It absorbs substances derived from the food or surroundings within the package, or it releases the chemical compounds into the food or surrounding environment like antioxidants, preservatives, and flavourings (EU 2009). And the released components are permitted to be used as food additives.

The application of active packaging-based systems in existing and recently developed food products is novel and ensures that food must reach the consumers retaining their original or enhanced sensory characteristics, with prolonged shelf life and safety which may helpful in reducing the food wastage (Dainelli et al. 2008). Still, the future of active packaging is strongly based on the cost-effectiveness and acceptance for both the consumers and the industry. The most important active packaging systems (adsorbing and releasing systems) used in food products include:

1. Oxygen scavengers/absorbers
2. Moisture absorbers/scavengers
3. Ethylene scavengers
4. Carbon dioxide emitters/scavengers
5. Flavour and odour absorbers or releasers
6. Antimicrobial packaging
7. Antioxidant packaging

Oxygen Scavengers/Absorbers

Oxygen, a key element for supporting the life, however, compromises the food shelf life resulting to degradative oxidation reactions and growth of moulds and aerobic bacteria which cause the quality deterioration by the production of odour, off-flavour, and harmful compounds. It can degrade the vitamin C present in some beverages like orange juice resulting to nutritional losses. Oxygen (O_2) when comes in contact between one and four electrons is reduced to a kind of intermediate compound forming superoxides, hydroxyl radical, hydrogen peroxide, and water among which all are very reactive (free radical in nature) except water resulting in oxidative reactions (Zenner and Benedict 2002).

The quality loss of oxygen-sensitive food products like milk powder, packaged pasta, biscuits, fruit juices, etc. can be reduced using the oxygen scavengers which alleviate the oxygen molecules left after the packaging process (Suppakul et al. 2003). Initially, self-adhesive labels or other adhesive aids and sachets were utilized for the development of oxygen-scavenging systems. However, main problems with the use of sachets are as follows:

- They need extra packing for keeping the sachets to every container/package.
- They cannot be added in case of liquid foods because they lose their activity when wet except ascorbic acid (Day 2008).
- Aqueous slurry of oxygen scavenger is formed in high-moisture foods when moisture introduces into the sachet. The slurry is then discharged onto the food product, ruining its appearance (Yeh et al. 2008).

However, nowadays oxygen-scavenging components are being included within the packaging material itself making use of enzymes, mono- or multilayer substances, and reactive closure liners for jars and bottles.

Commercially, oxygen scavengers are no doubt the utmost significant subcategories of the active packaging and used in the association with vacuum packaging or modified atmosphere packaging (MAP). Oxygen scavengers aid to reduce the permeation of oxygen through the package during storage, transportation, and retail practices. Fast-acting oxygen scavengers are highly efficient oxygen collectors and able to remove oxygen and work indefinitely till the scavengers are present. They are able to remove the oxygen to less than 0.01% as reported by some manufacturers (Vermeiren et al. 2003). This is important in processed meat products like fermented sausages or cooked ham where rapid discolouration may be caused if the package with traces of oxygen is exposed to the lighting causing the photooxidation processes (Coma 2008).

Generally, the oxygen scavengers work on the iron powder oxidation, but non-metallic oxygen absorbers have been recently developed for minimizing the ill-effects of metal-based scavengers like prospective health issues, causing arc while microwave heating, being detected in the metal detectors, etc. Organic substrates like catechol, ascorbic acid, and polyunsaturated fatty acids are easier to oxidize and introduced into the sachets, labels, and nowadays into the polymer blends (Lee 2014). Some microorganisms such as *Pichia subpelliculosa* and *Kocuria varians* have been included in the oxygen scavengers as an alternative to chemical scavengers which are getting the advantages of maintaining the sustainability. The spores of *Bacillus amyloliquefaciens* were incorporated as scavenger in the polyethylene terephthalate (PET) copolymer having 1,4-cyclohexane dimethanol which can be activated within 1–2 days under the high humidity at 30 °C after which it could efficiently absorb the oxygen for a minimum of 15 days (Anthierens et al. 2011). Also, the enzyme-based oxygen-scavenging systems are also developed where ethanol or glucose oxidase is fused into the adhesive labels or immobilized on the

Table 13.1 Commercially available oxygen-scavenging systems and their manufactures

Oxygen-scavenging systems	Manufactures	Packaging applications	Reference(s)
Oxy-Guard™	Clariant Ltd.	Muscle foods	Clariant (2017)
FreshPax®	Multisorb Technologies, Inc.	Muscle foods	EFSA (2014)
ATCO®	STANDA	Frozen foods	Laboratories STANDA (2017a)
Ageless®	Mitsubishi Gas Chemical Company, Inc.	Frozen foods	Mitsubishi Gas Chemical (2017a)
OMAC®	Mitsubishi Gas Chemical, Inc.	Meat and fish products	Mitsubishi Gas Chemical (2017b)
Cryovac® OS2000	Sealed Air Corporation	Cheeses, meat, baked goods, and dry products like nuts, coffee, and other snack foods	Sealed Air (2017)

film surfaces to retard the rancidity as well as oxidation reactions in the packaged food like chilled fish (Day 2003). It has been found that addition of unsaturated functional groups in the polymer films significantly improve their oxygen-scavenging power (Ferrari et al. 2009). Oxygen-scavenging systems comprising the nature-derived free radical scavengers such as ascorbic acid/α-tocopherol and a metal don't need to be activated using UV light which is otherwise needed if metal is used alone.

For commercial applications, the oxygen scavengers are usually introduced to the packaging materials to exclude the nonedible waste along with the food, reducing the uncertainty of sudden rupture or crack of the sachets in the package and consumption of their contents (Suppakul et al. 2003). Some of the commercially available oxygen-scavenging systems based on the sachet is given in Table 13.1.

Moisture Scavengers/Absorbers

In case of foods with high water activity, surplus moisture is formed within the package resulting in the bacterial and mould growth which leads to the reduction of shelf life and food quality. Therefore, moisture scavengers are necessary for controlling the moisture formation to prevent microorganism growth and improve the product appearance (Ozdemir and Floros 2004). After oxygen absorbers, moisture scavengers are the commercially developed category and available in various forms like pads, sachets, sheets, or blankets.

Desiccants like activated clays, silica gel, calcium oxide (CaO), and other minerals are generally porous and tear-resistant plastic-based sachets which are used to control moisture and humidity in case of dried food packaging. The best known moisture scavengers are conventional silica gels that can absorb approximately 35%

Table 13.2 Commercially available moisture-scavenging systems and their manufactures

Moisture-scavenging systems	Manufactures	Packaging applications	Reference(s)
Cryovac®Dri-Loc®	Sealed Air Corporation	Meat and fish packaging	Sealed Air (2017)
MeatGuard®	McAirlaid Inc.	Meat, fish, and soft fruit packaging	McAirlaid (2017)
Nor®Absorbit	Nordenia International AG	Microwavable susceptors and packaging	Nordenia (2011)
Fresh-R-Pax®	Maxwell Chase Technologies	Fresh-cut fruits	Maxwell Chase Technologies (2017)
TenderPac®	SEALPAC GmbH	Meat products	SEALPAC (2014)

of their own weight in water and maintain the water activity less than 0.2. On the other hand, molecular sieves like zeolites may pick up to 24% of their weight in water and also absorb odours, when dry. Nowadays, moisture drip-scavenging sheets, pads, and blankets are being synthesized by many companies to control the moisture in foods with high a_w like fruits, vegetables, meats, poultry, and fish. Such absorber typically consists of a double layer of microporous non-woven polymer like polyethylene (PE) or polypropylene (PP) which is located onto the super absorbent compounds like starch-based copolymers, carboxymethyl cellulose, and polyacrylate salts. These materials are commercially found in the form of different size sheets, employed as drip-absorbing pads and typically observed in tray configured either overwrap or in case of modified atmosphere packaging of muscle foods (Kerry et al. 2006). These drip scavenger pads are usually fixed below the packed fresh meat, fish, and poultry to suck in the hideous tissue drip exudate. Massive sheets and blankets are employed for air transportation to absorb the melted ice discharged from chilled seafood and control the transpiration in fruits and vegetables. These scavengers can also be used with (i) activated carbon to absorb the odour or (ii) iron powder to absorb the oxygen thus exhibiting dual action. Likewise, the double-action carbon dioxide (CO_2) or oxygen absorber sachets and labels are employed commercially for the foil packaged and canned coffee in the USA and Japan (Rooney 1995). Some commercially available moisture-absorbing pads are enlisted in Table 13.2.

Ethylene Scavengers/Absorbers

Horticultural produce releases the ethylene (C_2H_4), a ripening hormone, after their harvesting. Ethylene initiates and then fastens the respiration rate leading to senescence which softens the tissues, increases the degradation of chlorophyll, and decreases the shelf life of raw and minimally processed vegetables and fruits (Knee 1990). Therefore, control of ethylene accumulation while storage is critical to enhance the post-harvest life and to preserve the organoleptic quality of produce as well as processed products. In such cases, ethylene scavengers are employed to absorb the emitted ethylene and to preserve the ethylene-susceptible vegetables and fruits like mangoes, apples, onions, tomatoes, bananas, and carrots.

The most widely used and inexpensive ethylene scavenging system comprises of strong oxidizing agent potassium permanganate (4–6%) embedded onto the carrier (inert) having enormous surface area like alumina pellets, celite, vermiculite, silica gel, activated carbon, perlite, or glass for improved effectiveness (Zagory 1995). Potassium permanganate changes its colour from purple to brown upon oxidation, and therefore ethylene scavenging capacity is indicated by the colour change. Other kinds of ethylene scavenging system are dependent on:

1. Adsorption of ethylene either alone or in combination with other oxidizing agent. For example, palladium exhibits higher ethylene scavenging ability under higher relative humidity than the absorbers containing potassium permanganate (Smith et al. 2009).
2. Adsorption and then disintegration of ethylene into activated carbon. Such adsorbing technologies typically rely on the incorporation of precisely fine minerals like zeolites, Japanese oya, and clays into the films (Zagory 1995).

Ethylene scavenging systems are available in the market alone or combined with package. Retarder® and Ethylene Control Power Pellet are the commercially available ethylene scavengers based on potassium permanganate. They are available in the market as a sachet or inserted in the polymer as finely dispersed minerals. Ethylene absorption by zeolites placed in a polymer is found in PEAKfresh® and Evert Fresh Green Bags® scavenging systems (de Abreu et al. 2012).

Carbon Dioxide (CO_2) Scavengers/Emitters

Carbon dioxide (CO_2) is toxic to the growth of aerobes (bacteria or fungi) due to the decreased relative oxygen level and antimicrobial effects which result in the prolonged lag phase as well as generation period in the log phase during the growth of microorganisms. High carbon dioxide levels (nearly 10–80%) find applications in meat preservation for the inhibition of surface growth of microorganisms and thus increasing the storage life (Kerry et al. 2006). Hence, carbon dioxide emitters are considered like a supportive system to oxygen scavengers (Suppakul et al. 2003). Aerobic bacteria like *Pseudomonas* may be prevented by using the medium to high levels (10–20%) of CO_2, while the lactic acid bacteria reproduction may be triggered by the CO_2. Moreover, several pathogens like *L. Monocytogenes*, *C. botulinum*, and *C. perfringens* are partially inhibited by the concentration (<50%) of carbon dioxide. A study reported the increased production of *C. botulinum* at higher CO_2 levels while reducing the bacterial growth rate (Lövenklev et al. 2004). Hence, application of CO_2 packaging must be precisely scrutinized depending upon the various meat products and CO_2 levels.

The carbon dioxide scavenger removes the carbon dioxide from package headspace irreversibly resulting in depletion of CO_2 that is not always desired. An oxygen scavenger and carbon dioxide emitter can be used in combination for food products where appearance and volume of package are important because they prevent O_2 absorption resulting in the collapse of package. In modified atmosphere packaging (MAP), carbon dioxide usually has a microbiological inhibitory effect, but surplus levels of carbon dioxide may adversely affect the product (sometimes change the taste of product). Hence, it is necessary to remove the carbon dioxide in some packaging systems to ensure the food preservation. Calcium/sodium/potassium hydroxide, silica gel, and calcium oxide are widely used as CO_2 absorber to inhibit the package cracking (Ahvenainen 2003). At high water activity conditions, the calcium hydroxide combines with carbon dioxide to form the calcium carbonate:

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O$$

CO_2 emitters are applied mainly to decrease the gas to product (gas-product) volume ratio resulting to lowered headspace. They are commercially employed as the absorbent pads and sachets in meat, poultry, and cheese packaging (Realini and Marcos 2014). Several commercially available CO_2 emitters are based on the mixture of sodium bicarbonate (Na_2CO_3) and ascorbic acid or ferrous carbonate ($FeCO_3$) such as:

1. Verifrais™ from SARL Codimer, France
2. Ageless G from Mitsubishi Gas Chemical Co., Japan
3. FreshPax M from Multisorb Technologies Inc., USA

CO_2 Fresh Pads™ (CO_2 Technologies, USA) and SUPERFRESH system containing CO_2 emitter coated polystyrene box (Vartdal Plastindustri AS, Norway) are employed for the packaging of meat, poultry, and seafood to increase their shelf life and reduce the environmental impact and transport volume (Realini and Marcos 2014). In the case of CO_2® Fresh Pads, the mats absorb the liquids exuded from the food products, and liquids combine with the sodium bicarbonate and citric acid to produce carbon dioxide.

Flavour and Odour Absorbers or Releasers

The incorporation of odours and flavours is basically employed to make the food more appealing to the consumers and enhance the aroma or flavour of fresh food or processed product on opening the package. Such aromas and essences are generated gradually and uniformly inside the packed product during its storage life, or their spread may be regulated while food preparation or opening the package. Slow distribution of aroma can be used to balance the inherent loss of smell or taste of food during the entire storage life (Almenar et al. 2009).

On the other hand, the odour removal from the package interior can be detrimental and advantageous also. In earlier case, the absorption of aroma components can withdraw the desired constituents from the product because occasionally the aromatic compounds are naturally accumulated in the interior of the package like in the case of orange juice. In such conditions, the loss of desired aroma must be prevented from the food product which is also one of the objectives of the barrier packaging. However, the removal of odour or aroma is beneficial several times under the domain of active packaging. Some food like cereal products and fresh poultry produce the particular odour called as confinement odour. Some off odours like sulfurous compounds obtained from the breakdown of protein/amino acid or aldehyde/ketone constituents obtained from the lipid oxidation or anaerobic glycolysis are sometimes formed in very less but yet detectable concentration during the product distribution. Such odours are confined within the gas barrier packaging, and they are released when the package is opened and detected by consumers. These odours don't necessarily mean any major food spoilage and are usually harmless but still are rejected even they dissolve into the surroundings. The major cause for their removal from package interior is to prevent or eliminate the potential side effects of these confinement odours. Other possible reason for introducing odour scavengers may be to remove the effect of odour produced in the package materials.

Antimicrobial Packaging

Antimicrobial packaging means the package itself possesses the self-sterilizing capability and thus exhibits a critical role in the packaging of perishables such as meat, fish, poultry, and horticultural produce because they provide all the nutrients required for the microbial growth. This packaging thus helps in reducing the multiplication of pathogenic microbes like *L. monocytogenes*, *Salmonella* spp., *S. aureus*, *C. botulinum*, *C. perfringens*, and *E. coli* O157:H7 to enhance the shelf life and confirm the delivery of safe wholesome food to final consumers (Jayasena and Jo 2013). It lengthens the lag phase and minimizes the log phase of the growth cycle of microorganisms to preserve the food. Basically, antimicrobial packaging can be categorized into four classes as:

1. Direct introduction of antimicrobial compound(s) into the polymer film
2. Addition of antimicrobial pad/sachet inside the package
3. Use of innately antimicrobial polymer
4. Coating the packaging film with a matrix

Several antimicrobial compounds comprising carbon dioxide, ethanol, chlorine dioxide, silver ions, antibiotics, organic acids, peptides, bacteriocins (nisin), spices, plant extracts, and essential oils were examined to inhibit the growth of microbes in foods (Suppakul et al. 2003). The effectiveness of antimicrobial packaging is affected by various factors like:

1. Choosing an effective delivery method
2. Selection of suitable antimicrobial substance
3. No/negligible effect on the organoleptic characteristics of packaged product

Nowadays, lactic acid bacteria-based bacteriocins which are basically hydrophobic, cationic, and amphiphilic peptides are widely used as an antimicrobial agent mainly to Gram-positive bacteria. Antimicrobial packaging using nisin is being utilized to inhibit the multiplication of lactic acid bacteria on the beef burger kept at 4 °C and thus enhance its shelf time (Ferrocinoa et al. 2016). Nisin exerts synergistic effect when used with other preservatives. Nisin in combination with potassium sorbate produces the synergistic effect against *Listeria innocua* and *Zygosaccharomyces bailii* in vitro when added in tapioca starch hydroxypropyl methylcellulose films (Basch et al. 2013). A study conducted by Jofré et al. (2008) reported that sliced cooked ham when vacuum packaged using interleavers carrying nisin (200 AU/cm^2) and potassium lactate (1.8%) was more strongly protected against the growth of *L. monocytogenes*. However, the major limitation regarding the commercial use of nisin extract is the low concentration (e.g. 2.5%) of nisin (Royal DSM, Delvo® Nis, Nisaplin®, and Danisco) which means a huge quantity of extract is required to get the desired antimicrobial effect. Additionally, various semi-purified bacteriocins are also used as an antimicrobial agent because bacteriocins are basically the natural compound obtained from the bacterial growth. Enterocins obtained from *Enterococcus faecium* are applied in meat products to regulate the growth of *L. monocytogenes* (Marcos et al. 2007). Lactocins when used as an antimicrobial film decreased the growth of *L. innocua* on the surface of wiener sausages (Blanco et al. 2014). Most recently, live bacteria can be employed to introduce the bacteriocins into antimicrobial packaging. Further, bacteriocin-containing packaging films may be combined with the novel processing technique such as high pressure processing (HPP) to decrease of microbes more effectively. Laboratories STANDA has developed SANICO®, an antibiotic (natamycin)-based antifungal coating employed for sausages (Laboratories STANDA 2017b). The nisin/polylactic acid (PLA) films were developed by Jin and Zhang (2008) for their use either as packaging material for bottles or coating on the surface of bottles to minimize the microorganism's proliferation in fruit juice packaging.

Nanotechnology can improve the antimicrobial packaging using nanoparticles which possess the increased area than traditional antimicrobial compounds and thus reduce the quantity of antimicrobial substances because of magnified antimicrobial activity of nano-compounds. The cellulose/silver nanocomposites reduced the harmful moulds and yeasts up to 99.9% in kiwi and melon juices which verified the antimicrobial activity of silver-based nanoparticles (Lloret et al. 2012). A wide range of silver-based antimicrobial master batches can be seen in the market like Agion®, Bactiblock®, Biomaster®, d2p®, IonPure®, Irgaguard®, and Surfacine®. Addmaster and LINPAC Packaging Ltd. joined hands together for the development of antimicrobial trays and lids with the Biomaster®, a silver-containing additive so as to retard the multiplication of pathogenic organisms like *Salmonella*, *Campylobacter*, and *E. coli* in the fresh meat (LINPAC 2012, 2017). Sodium alginate film which carries zinc oxide nanoparticles removed the *Staphylococcus aureus* and *S. typhimurium* in RTE poultry meat (Akbar and Anal 2014).

Further, a new trend of phenolic compounds' incorporation to essential oils like thymol, eugenol, and carvacrol has attracted huge attention for possessing natural antimicrobial activity against meat-based products (Jayasena and Jo 2013). The basic mechanism behind these compounds' antimicrobial action is the increase in permeability of microbial cell membranes which causes the loss of cellular ingredients. Recently, the encapsulation of essential oil combined with other components like nisin, MAP, and lysozymes into the nano-emulsion are being used in the meat sector to improve the safety and organoleptic properties of meat and other processed products (Jayasena and Jo 2013).

Antioxidant Packaging

Oxygen in excess concentration may promote the lipid oxidation especially in animal products, microbial growth, and development of nutritional losses, colour changes, and off-flavours/odours. The oxidation of lipids leads to the rancidity development and forms the poisonous aldehyde compounds thus deteriorating the nutritional quality due to the degradation of polyunsaturated fatty acid (PUFA). Likewise, various oxidative reactions in the food products are the main cause for quality deterioration. Therefore, the antioxidant agents can be incorporated in the package to remove the oxygen for increasing the storage life, besides using oxygen scavengers. Antioxidant packaging has several following advantages than the direct addition of antioxidants:

1. Very low amount of active compounds needed
2. No need of additional steps like spraying, mixing, or immersion
3. Modulated antioxidants' release
4. A confined activity

However, the addition of antioxidants can influence the various quality attributes like taste or colour, and thus the consumer preference is required for the exclusion of additives in foods.

Several antioxidant compounds are introduced successfully in diverse forms like labels, coating, and sachets, and are added into the polymer matrix or immobilized on polymer surface in the package to prevent oxidative reactions occurring in food. Recently, the major trend is to use natural antioxidant compounds like tocopherol, ascorbic acid, and herb-derived essential oils like tea, rosemary, oregano, and plant extracts thus minimizing the use of synthetic additives. The blue shark muscles packed in the polymer film containing the barley husk-derived natural antioxidant had exhibited the reduced oxidative degradation (de Abreu et al. 2011). Similarly, the lipid oxidation was decreased to 80% in beef packed with films carrying the natural extract obtained from residual waste of brewery (Barbosa-Pereira et al. 2014). Cocoa extract carrying active films have been reported by Calatayud et al. (2013) which possesses both antioxidant and antimicrobial characteristics. Nowadays α-tocopherol a natural antioxidant has been added into polylactic acid

(PLA) films (a versatile compostable polymer) as an antioxidant packaging material (Jamshidian et al. 2012). Recently extensive research is being conducted for combining the antioxidant packaging with other novel processing treatments like HPP to improve the food quality and safety (especially meat-based products) (Marcos et al. 2008).

The antioxidant action of polymers is based on the migration process where the components released must meet the maximum allowable concentration and must be permitted as food additives. However, antioxidant packaging systems may be best effective when antioxidant release rate would be fitted with the rate of lipid oxidation (Lee 2014). The diffusion-based mathematical models can be a suitable technique to determine the profile of antioxidants' release into the food and beverage. But more research work is necessary to regulate the rate of bioactive agents' diffusion in the practical packaging systems while on storage.

Other Active Packaging Systems

Ethanol emitters are another category of active packaging systems which suppress the growth of bacteria and yeasts and are mainly effective against mould. Ethanol emitters are mainly employed in the sachet form extensively for high-moisture bakery products like cakes and cheese to make them mould-free and extend the shelf life by 2000%. In food packages containing sachets of ethanol emitters, the water molecules are absorbed by the food; ethanol vapours are released and thus diffused into the package headspace (Day 2003).

Temperature-controlled active packaging systems utilize the novel self-cooling/heating cans and insulating substances to reduce or eliminate the disproportionate temperature abuse while on transportation as well as storage of frozen and chilled foods. One such kind of insulating material is Thinsulate which contains several air openings. The chilled temperature can be regulated by increasing the package thermal mass which enables the package to tolerate the temperature increase. Self-heating containers and cans consisting of aluminium and steel are heated using an exothermic reaction which takes place on the mixing of lime and water positioned in the base and have been available commercially from decades for sake, tea, coffee, and ready meals mainly popular in Japan. The latent heat produced during water evaporation creates the cooling effect and this principle was exploited by CROWN Packaging Europe GmbH, Switzerland, in association with Tempra Technology, Inc., Florida, to develop a self-chilling beverage can (Tempra Technology™ 2017).

Nowadays, the convenient foods are high in demand due to which the microwave heating has become a trend in food outlets and household. However, heat transfer in microwave heating produces the varying energy absorption which results in the irregular distribution of temperature in the food products. Therefore, the microwavable active packaging aims to improve the heating performance of food using the field modification, susceptors, and shielding (Regier 2014). The modifiers comprised of a sequence of antenna structures thus altering the path through which microwaves reach to the food resulting in uniform crisping, heating, and surface browning (Ahvenainen 2003). Shielding may be used to obtain more uniform heating and regulated distinct heating of several food segments. On the other hand, microwave susceptors are made of stainless steel or aluminium accumulated on the substrates like paperboard or polyester films and used to crisp, dry, and finally brown the microwave food (Perry and Lentz 2009). Commercially available microwave susceptor is Sira-Crisp™susceptors (Sirane Ltd.) (Sirane 2011) whose application lies in the heating of hot dogs, sandwich, frozen entrees, or meat pies as the meat-containing foods.

Organoleptic alterations in the food products are due to the intended or non-intentional interactions between the polymer and product and sometimes due to the unsuitable medium characteristics essential to protect the food quality. In such kind of case, taste and smell emitters are the fascinating solution. The emitters of smell uniformly spread the scent masking fragrant compounds in the packaging systems and improve the natural aromas of the product and attract the consumers to buy the product again. Such compounds are identified by the very low thermal conductance and used as additive to polyamide, polyester, polyethylene, polypropylene, and polyvinyl chloride.

Intelligent Packaging

The intelligent packaging may be defined as "the packaging system which performs the multiple intelligent tasks like identifying, tracing, sensing, recording and communicating the information to increase the safety, quality, shelf life, and alert regarding the possible problems". An intelligent package traces the product, senses the external/internal conditions of the package, and informs the consumer about product health thus monitoring the quality and safety of food and beverage, while active packaging system takes some action (like scavenge the oxygen or moisture) to protect the product. An intelligent packaging system contains small and inexpensive smart devices either tags, sensors, or labels that acquire, store, and transfer valuable information regarding characteristics and functions of food products. The frequently employed smart devices for such kind of packaging are discussed as:

Barcode

The barcode is an optical machine-based comprehensible symbol and strongly concerned to the device being attached. The universal product code (UPC) was established in the 1970s and was the first successfully commercialized barcode which now has omnipresence in the grocery stores for effective stock reordering, inventory control, and checkout. The UPC barcode has a straight indication comprising of specific arrangement of spaces and bars for expressing the 12 numerals of data carrying particular and confined data like item number and manufacturer identification number (Yam et al. 2005).

Recently, innovative barcode symbologies like two-dimensional (PDF 417, Aztec code), Composite Symbology (a 2D barcode like PDF 417 containing a straight barcode such as UPC), Reduced Space Symbology (RSS), and Family of GS1 DataBar have been developed to meet the emerging need to encode more data in very small space (Uniform Code Council 2017). Information about lot/batch number, packing date, package weight, nutritional information, how to use, and website address of manufacturers can be encoded in barcodes. This information is even readable by the smart phones thus offering great convenience for retailers and consumers.

Radio-Frequency Identification (RFID)

RFID technology also carries the electronic information like barcodes but is more advanced data carrier for product identification having various novel properties like huge data storage capacity (for high-end RFID tags up to 1 MB) and non-line of sight capability, while collection of the real-time data and data may access the non-metallic substances for automatic and fast identification of the multiple product (Mennecke and Townsend 2005). This technique employs tags attached to any package/cattle/pellets, etc. to communicate real-time correct information to the receiver's information system. It has several advantages in the stock management, traceability of product, labour saving expenses, security and improvement of safety, and quality. However, RFID tags are not exactly the substitute of barcode due to its comparatively higher cost and requirement for more strong and efficient electronic information network.

The RFID tag consists of a very small transponder and antenna with distinctive alphanumerical or number sequence. A reader releases the radio wave to record the data from the RFID tag which is then transferred to the host computer using real-time database server for decision-making and analysis. The real reading limit is based on several factors like strength of reader, frequency of operation, and potential intervention from various metal objects (Yam et al. 2005). The tags with low frequency, about 125 kHz, are usually economical, have better penetration through non-metallic items, and consume less power.

Advances in RFID technology involves the combination of TT sensors and RFID devices resulting in the improvement of food supply chain and increased funds because of the low generation of waste. Few reusable TT sensor tags which were developed for providing the information about the history of product based on the real-time temperature through cold chain include the Easy2log© (CAEN RFID Srl), Sensor Tag CS8304 from Convergence Systems Ltd., and also TempTRIP Sensor Tag from TempTRIP LLC (CAEN RFID 2017; CSL 2017). Recently, the RFID tag integrated along with the optical oxygen indicator containing a platinum octaethylporphyrin membrane and a complete electronic-based system had been invented, and interestingly all were printed on the flexible substrate. This whole system was ideal for use in MAP applications in which oxygen level is lowered to less than 2% (Martínez-Olmos et al. 2013). Georgescu et al. (2008) employed the acoustic wave devices in the RFID system including a ID, an electronic module, various passive surface acoustic wave sensors, and printed antenna which observes several chemical and physical parameters of contents through the food supply chain. Sen et al. (2013) reported a monitoring system which consisted of temperature sensor, RFID tag, gas sensor, reader, and server to determine the freshness of pork. RFID has found great application in the packaging of meat-based products where tags may be concealed by meat and thus suitable for scanning of products with high moisture content. Recently, it is being adopted by several dominant companies like Marks & Spencer, Walmart, and 7-Eleven.

Time-Temperature Indicators (TTI)

The time-temperature integrator/indicator (TTI) is an easy-to-use, simple device affixed to the individual package or shipping containers as self-adhesive label which monitors, records, and displays the quantifiable time-temperature-based changes representing the partial or full temperature record of a food product mainly frozen or chilled throughout the food chain thus ensuring product quality and safety.

The fundamental principle of TTIs is to identify irreversible responses of chemical, electrochemical, mechanical, microbiological, or enzymatic changes in the food product at higher temperatures (Kerry et al. 2006). Further, the response level (real time-temperature history) strongly relies on the type of indicator and its working principle. Commercially, there are three types of TTIs based on the response mechanism where irreversible and temperature-dependent enzymatic, chemical, or microbiological changes make the response:

1. Critical temperature indicator which indicates the exposure of product below or above reference temperature
2. Partial history indicator that shows the exposure to a temperature enough to affect the food quality or safety
3. Full history indicator which communicates the consistent response based on the temperature during the history of the product

Currently, some marketed TTIs include CheckPoint, 3M MonitorMark ®, ColdSNAP Temperature Recorders, ShockWatch, ThermRF Logger, Fresh-Check®, Timestrip®, VarioSens®, WarmMark Time-Temp tags, and many more. 3M Company uses a TTI called 3M Monitor Mark® which consists of a mixture containing the esters of fatty acid containing the preferred melting point as well as blue dye. When temperature reaches above the critical value, the substance starts melting and diffuses via the indicator making the blue colour to visualize.

TTIs can be combined with RFID tags or barcodes to generate simpler and efficient time temperature history that can be associated with other food products' data. A TTI Smart Barcode, FreshCode™, is one such device which contains standard barcode as well as senses and stores the temperature abuse through the cold chain. A time-temperature indicator based on the lactic acid was produced by Wanihsuksombat et al. (2010) to observe the food quality where diffusion of lactic acid causes colour changes (from green to red) due to pH reduction and temperature dependency which was identified from 4 to 45 °C. VITSAB® (VITSAB International AB) is TTI dependent on an enzymatic reaction in which alteration of colour from green to clear yellow takes place because of enzymatic hydrolysis of the substrate (VITSAB 2015). Ciba and Freshpoint™ jointly launched the OnVu™ which contains a pigment called benzopyridines which change the colour at temperature-based rates with time. The indicator turns to the dark blue colour when exposed to UV light and starts to fade the colour slowly (Freshpoint 2017b; O'Grady and Kerry 2008).

Gas Indicators

Gas indicators are the devices which can be printed on the polymer films or exist as a label to sense any alternation in the make-up of gas mixture due to package nature and activity of the food like respiration and gas production by growth of microorganisms thus monitoring the food quality, safety, and integrity. Gas indicator causes the change in colour on the packaging to show the gas composition changes, quality degradation of MAP foods, and spotting the poor sealing. A commonly used gas indicator is oxygen indicator due to its side effects on quality of food through oxidative rancidity, microbial spoilage, and colour change. The UV light-activated reusable and irreversible oxygen indicators had been developed by Lee et al. (2008) that consisted of a mixture of an encapsulating polymer like hydroxyethyl cellulose, a redox indicator-like methylene blue, UV-absorbing semiconductor like TiO_2, and an electron donor like triethanolamine; this mixture is being mixed with water to produce ink. The coating/printing of ink on substrates turned them to colourless oxygen indicator film from being blue-coloured on the exposure to UV light. This colourless film is then reoxidized to the original blue colour when it came in contact with oxygen. Likewise, the oxygen indicator named Ageless Eye® has been developed by the Mitsubishi Gas Chemical Company that can be placed inside the container. If oxygen level will be more than 0.5%, the indicator turns its colour from pink to blue. The simple UV-activated inkjet-printed oxygen indicator was reported by Lawrie et al. (2013). Nano TiO_2 powder when mixed with oxidation-reduction dyes, electron donors, and packaging polymers had the potential to trace the oxygen concentration in MAP meat products (Liu et al. 2013).

Further, the gas indicators for other gases like CO_2, H_2S, ethanol, water vapour, and several supplementary gases have been found in literature. The CO_2 indicator is made up of redox indicator dye and calcium hydroxide ($Ca(OH)_2$) incorporated to the polypropylene (PP) and employed for the estimation of fermentation level in kimchi products during supply chain (Hong and Park 2000).

Freshness Indicators

Freshness indicators are the devices showing the degradation and loss of freshness of packaged goods and more beneficial for quality control of packed fruits and meat products. These are dependent on the traditional knowledge of food quality which shows the metabolic products such as organic acids (especially lactic acid), glucose, volatile nitrogenous components, ethanol, biogenic amines (like cadaverine, tyramine, histamine, putrescine, etc.), ATP degradation products, CO_2 and sulfuric compounds linked with spoilage flora, type of meat products, storage conditions, and packaging type. Most of freshness indicators work via colour change in the substrate because of the presence of abovementioned compounds during loss of freshness of products.

The chitosan film containing anthocyanin was employed to develop the colorimetric pH indicator that is used to indicate the formation of microbial growth metabolites like lactic acid, D-lactate, acetic acid, and n-butyrate (Yoshida et al. 2014). In the case of fish, the volatile amines may be used as freshness indicator because trimethylamine oxide degrades into the volatile amines and produce fishy odour and flavour (Etienne and Ifremer 2005). The optoelectronic nose comprising the seven perceiving compounds is manufactured using the chromogenic reagents as well as pH indicators and is employed in the monitoring the pork sausages quality (Salinas et al. 2014).

The recent emerging solution for supervising the food freshness is to develop and use the biosensors for the determination of specific metabolites which are originated during the food spoilage. A biosensor was developed by Pospiskova et al. (2012) to detect the traces of basic nitrogen compounds and biogenic amines produced during microbial metabolism. Further, biosensors can be integrated to the packaging polymer using molecular imprinting technology to generate the recognition compounds for specific analyte molecules which are introduced in prepolymeric mixture to bond with prepolymer. Then, prepolymeric mixture is polymerized using analyte molecule to produce the polymer with the removal of analyte molecules at the end leaving the analyte molecule's shape cavities behind. Like this the specific compound is detected as the cavity shape is particular to the modelled molecule. Thus, an inexpensive packaging material was invented to show the spoilage of meat using the variations in colour (Johns Hopkins University Applied Physics Laboratory 2014).

However, the major limitation with commercialization of freshness indicators is the reluctance of food processors to utilize these indicators for determining freshness because this may harm their image in market, if products are not fresh.

Pathogen Indicators

Pathogen indicators are usually the biosensors which detect, record, and show the information about the biochemical reactions or simply contamination by the pathogenic microorganisms. These are composed of a bioreceptor (biological or organic compounds like antigen, microbe, enzyme, hormone, or nucleic acid) that identifies the target analyte and a transducer (electrochemical, optical, or calorimetric) which produces measurable electrical response from the conversion of biochemical signals and changes the colour to warn the consumers.

A sensor consisting of cross-polymerized polydiacetylene molecules may react with toxin like *E. coli* O157 enterotoxin to change the blue colour of film permanently to red (Smolander 2000). Food Sentinel System™, a commercially used pathogen indicator, has been developed by SIRA Technologies, USA, in which an antibody particular for the target pathogens like *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* sp., etc. associated with the membrane forming a part of barcode along with pathogen organisms if present generates a confined dark bar making it difficult to read the barcode on scanning. A pathogen indicator named Toxin Guard™ was launched by Toxin Alert, Canada, which was composed of biochemical sensors introducing the antibodies to trace the pathogens like *E. coli*, *Listeria* sp., *Salmonella* sp., and *Campylobacter* sp. in polyethylene (PE)-based packaging (Bodenhamer 2000). A novel packaging employs the vanillin, a colorimetric reagent, to detect the microbial growth in several products via visual signal without any direct contact between detection system and microorganism or product (De La Puerta et al. 2010).

Integrity Indicators

The time indicators are the simplest integrity indicators which contribute useful data regarding the duration for which a food product has been opened. These indicators in the form of label activate on breaking the seal by triggering a timer and changing the colour with the span of time. Commercially used integrity indicators are Novas® Embedded Label developed by Insignia Technologies Ltd., Best-by™ from FreshPoint Lab and Timestrip® launched by Timestrip Ltd. (Insignia Technologies 2017; Freshpoint 2017a; Timestrip 2016). Zhai (2010) invented the “voice advertisement” intelligent packaging which carries a tiny battery, voice chip-integrated circuit, and loudspeaker. The music or vocal comments regarding product information/state was played on opening the package thus prohibiting forgery and modifying the purchase/dining experience of consumers.

Further, various gas indicators discussed above also work as integrity indicators because they may provide information about any leakage influencing the integrity.

Bioactive Packaging

Bioactive packaging may be defined as "a novel packaging technique where the bioactive/functional packaging materials hold the required bioactive compounds possessing functional properties at optimum level till they are emitted within the package throughout the storage or before consumption to improve the consumer's health". It is different from the active packaging techniques in the way that active packaging is mainly concerned with maintaining or enhancing the quality together with safety of food and beverage, whereas bioactive packaging makes the packaged foods healthier and thus is directly related to the consumer's health. Several techniques which maintain the unique characteristics of biopolymers include microencapsulation, nanoencapsulation, enzyme encapsulation, and enzyme immobilization.

The bioactive packaging can be performed using the:

i. Regulated discharge of bioactive components from sustainable/biodegradable packaging systems
ii. Nano- and microencapsulation of bioactive agents in the packaging materials and also within food products
iii. Enzymatic activity to improve the health through transforming particular food-borne components (Lagaron 2005)

The biodegradable especially edible and sustainable matrices such as biomass-obtained thermoplastics, biodegradable polymers, emerging nano-biocomposites, polysaccharides and their derivatives, proteins and their derivatives, and smart biopolymers from microbes must be developed for the intact and prolonged shelf life integration and regulated release of bioactive compounds. Phytochemicals, vitamins, prebiotics, and nanofibers are the ideal functional components for integration in the package to promote the health. For the successful incorporation of bioactive substances, the following factors are necessary to obtain the required release rate as soon the package is opened and before its consumption:

i. Fabrication method of the films
ii. Optimal temperature/time combination for mixing bio-based packaging materials and functional substances
iii. Suitable packaging material
iv. Engineering mechanism

Encapsulation incorporates the enzymes, cells, food ingredients, or any other material in small capsules which protects them from moisture, cold/heat, or other adverse environmental situations increasing their stability and maintaining viability. Protein, fats, dextrins, starches, alginates, and various lipid compounds are used as encapsulating agents. The bioactive ingredients are released from the capsule using suitable methods like solvent activation which are site- and stage-specific and signalled using alteration in temperature, osmotic shock, irradiation, or pH (Lopez-Rubio et al. 2006). The application of enzymatic activity in transforming food is an emerging concept and is the most suitable and simple technique for enzyme immobilization is the encapsulation. The selection of suitable immobilization technique and biomaterial support to manufacture the enzymatic packages can be strongly based on the:

i. Characteristics of biocatalyst such as purified enzymes or whole cells derived from bacterial or fungal origin
ii. Expected storage constraints
iii. Kind of packed food
iv. Particular utilization of the biocatalyst

Nano-packaging

The nanotechnology, a science of tiny materials, is employed in food packaging to inhibit the food spoilage, maintain the quality, enhance the shelf life, and ensure the wholesomeness of food products and beverages. It may also assist the customers to improve the food as per their taste requirements and nutritional demands. A very minute amount of nanoparticle is enough to transform the packaging materials without significantly affecting their transparency, density, packaging, and processing properties because of a large aspect ratio of nanoparticle. Nanotechnology modifies the structure of any packaging material at molecular level.

Nanoscale-based innovation offers the novel modifications to food packaging by (i) improving barrier as well as mechanical properties, (ii) detecting the pathogens, and (iii) active/intelligent packaging thus exhibiting the food quality and safety advantages. The biodegradable films obtained from natural polymer have limited application in the packaging due to the inferior barrier and mechanical behaviour against the temperature control, carbon dioxide, oxygen, flavour and volatiles, moisture stability, and UV-blocking features as exhibited by the natural polymers, which can be improved using nanocomposites. Nanocomposites lower the packaging waste linked with processed products and preserve the fresh foods extending the shelf life. The packaging materials, bottles and other heavy packages by adding nanoparticles can be turned into lighter which possess resistance to fire and have stronger mechanical and thermal properties.

Nanocomposites

Most of nanocomposites are being developed for the beverage packaging due to their outstanding advantages over traditional packages. They modify the primary characteristics of packaging materials like strength, barrier properties, antimicrobial nature, and stability to heat/cold. Maximum work about the nanocomposites has focused to use montmorillonite clay (1–5% by weight) as a nanoscale component (must have one dimension <1 nm) in several polymers like polyethylene, polyvinyl chloride, nylon, and starch. Various methods like solution method, in situ, and melt processing method are employed to process nanocomposites. Nanocomposite plastic film and coating known as Durethan was produced by Bayer which is composed of clay nanoparticles spread uniformly across the plastic. Such nanoparticles block the path of moisture, oxygen, and carbon dioxide to make contact with content and thus prevent the diffusion process completely thus increasing the shelf life along with improving the quality (ETC Group 2004).

Beer can be now packed in plastic bottles using nanocomposites by Nanocor, subsidiary of Amcol International Corp, and have 6 months shelf life, earlier which was not possible due to flavour and oxidation problems. Research work has been undergoing to enhance the life of beer when packed in the plastic bottles containing nanocrystals up to 18 months by reducing the carbon dioxide loss from the bottle and restricting the oxygen entrance. Further, nanocomposite bottles are very light in weight thus lowering the distribution cost (ETC Group 2004).

Alternative Nanotechnology-Based Techniques

The carbon nanotubes are basically the cylinders containing the nanoscale diameters and used to improve the mechanical properties of package and also possess the antimicrobial activities. The cells of *E. coli* were punctured quickly on contact with the thin carbon nanotubes inducing the cellular injury (Kang et al. 2007a, b).

The nano-wheels developed by self-assembling of inorganic alumina platelets may be introduced into plastics to enhance their mechanical and barrier characteristics.

The food packages containing nanosensors detect the nutrient content of product as well as its quality. The nanosensors can find the toxins, chemicals, and microorganisms produced by the food. The biosensor made of poly (dimethylsiloxane) chips containing antibodies adhering to *Staphylococcus* enterotoxin B has an identification limit of 0.5 ng/mL. The nanovesicles can detect the pathogens like *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7. Moreover, the liposome nanovesicles may trace the peanut allergen proteins (Doyle 2006). NanoBioluminescence spray, with commercial name BioMark developed by AgroMicron, contained the luminescent protein that was adhered to the surface of microorganisms thus emitting visible glow varying in intensities based on the level of contamination (Joseph and Morrison 2006). Carbon nanotubes coated with DNA strands may be used to produce nanosensors to trace any odour and taste where the single DNA strand can be considered as a sensor and the carbon nanotube as a transmitter. Alike techniques are used in the synthesis of electronic tongue nanosensors to find out the compounds in parts per trillion thus warning the consumers on food spoilage. pSiNutria has developed nano-tracking technology which includes ingestible BioSilicon chip to monitor and detect the pathogens (Miller and Senjen 2008). Kraft had developed the nanosensors in association with Rutgers University that warns the consumer by colour change if food starts to spoil/degrade or has been fully spoiled using the electronic noses/tongues to taste or smell the flavours and scents (Sozer and Kokini 2009).

Nano-coatings are usually the waxy coatings employed for several products such as cheese, candies, chocolates, meats, fruits, vegetables, and bakery products which are strong barriers to the water vapour/moisture and gas. Recently, scientists have developed nanoscale edible film coating with a thickness of 5 nm which is not visible to the human eye. An edible antibacterial nanocoating was developed by the Sono-Tec Corporation, USA, which is useful for bakery products directly (El Amin 2007). Extensive research has been going on to develop nanoscale-based dirt-repellent coating.

Nanotechnology offers great advantages to the antimicrobial packaging by employing nanosilver which has antimicrobial activity at nanoscale and therefore can be incorporated in packaging polymers resulting in nanosilver composites for food preservation and extending the shelf life. Silver nanoparticles can reduce the 24 h bacterial growth to 98%. Nanocopper oxide, nanomagnesium oxide, and nano-titanium dioxide are also found to contain antimicrobial effects (Doyle 2006).

Responsive Packaging

Responsive packaging is the recent technology in packaging and can be defined as "the packaging system where package communicates due to the specific change in the package, food, headspace or external surrounding and emits encapsulated nutrients or active ingredients under specific environment to enhance the shelf life and quality of food, provide more convenience and theft resistance throughout the supply chain thus improving the packaged product safety". Responsive packaging system reacts only to stimulus present inside and outside of package where stimuli can be anything which adversely affects the food like food-borne threats, bacteria, moulds, contaminants, pH, moisture, or gas levels in the headspace. The direct contact between quality indicator/sensor and headspace or food product is necessary for providing the response about the food quality (Brockgreitens and Abbas 2016). Responsive materials such as self-assembled nanoparticles, hydrogels, layered films, supramolecular substances, and surface-grafted materials must be added into the packaging system which shows changes in chemical or physical characteristics in response to stimuli (Zelzer et al. 2013). The responsive food packaging can reduce the cases of food-borne diseases as seen in real time and also lower the food waste because fresh food is easily identified by consumers and processors (Gunders 2012). Responsive food packaging can be bioresponsive, chemoresponsive, thermoresponsive, and mechanoresponsive in nature based on the stimuli present in food or package.

No doubt, the responsive packaging is a kind of revolutionary technology for packing the food in the safest manner; however, various criteria must be taken into consideration before its commercialization. The performance of responsive materials must be clearly defined in terms of detection limit, sensitivity, working conditions, and range. They must exhibit reproducible response and should not display wrong indication of spoilage. This technology costs high, but its advantages in terms of improved safety and quality of food must be clearly presented to customers for satisfying them to paying extra money.

Microwavable Food Packaging

Microwave packaging aims at convenience and simply saves the time also. It also enhances the research and developments in novel microwavable food products. It regulates how uniformly and quickly a food heats. Moreover, the package may also provide the heated surface for creating high-heat steam environment for moisture retention, crisping, and browning (Regier et al. 2016). Earlier Al-foil-based containers were used widely, but many limitations like triggering microwave fires, arcing, magnetron destruction, and preventing microwaves penetration into food were observed. Susceptors are used as heating components which respond to the microwaves and thus regulate the heating rate to improve the cooking performance and prepare food like popcorn and frozen pizza crispy. Currently, the commercial susceptors are composed of metallized plastic films.

Recently, trays laminated using a polyester film of thin patterned aluminium were developed where patterned Al is employed to regulate and utilize the microwave energy. It distributes the microwave energy via specific Al patterning, transmitting it into frozen food to greater depth thus heating the food more uniformly and fast. Moreover microwave packaging employs strong sensors, digital displays, fuzzy logic, and other attributes improving the microwave cooking. The packages of the next generation communicate the consumer pr processor when you uncover, stir, add salt, etc.

Edible Packaging

Edible packaging makes it possible to eat food products on the retail counter along with their edible skins. It may reduce the food and packaging waste and migration of chemicals from the package to food. Edible packaging films and coatings are typically composed of proteins, carbohydrates, or fats based on their use. The substances for edible packaging must be considered like the additives when they have the edible purposes. Edible package must possess the necessary fundamental functional characteristics to act as a moisture barrier, and gases and novel blends or composites may be formulated for regulating the delivery of nutrients together with food additives (Campos et al. 2011). Currently, the edible packaging innovation lies in the following five categories in the food industry:

1. Food packed in an edible/biodegradable package
2. Food contained in food
3. A container or cup to be consumed with its beverage
4. Package that disappears
5. Edible packaging at quick-service restaurants

Edible packaging, one of the emerging technologies, supports the utilization of biodegradable polymers and sustainability and replaces the artificial compounds with natural ones. Further, it can be used as a medium delivery of functional or bioactive components, but diffusion of such compounds must be regulated. Further nanoscale structures may be incorporated in edible packages to enhance their applications; however full safety assessment is required before addition.

Recent Innovations in Packaging Materials

Glass containers are usually more resistant to several degradation factors in comparison to polythene and galvanized container. But the more oxidative stability is offered by brown container regarding physicochemical characteristics of the sunflower oil. The brown colour protects the contents from light which otherwise degrades the vitamins and pigments in the vegetable oils (Abdellah and Ken 2012).

The features of PET such as excellent clarity, UV resistance, mechanical properties, and good oxygen barrier characteristics make it the best choice in packaging of liquids that can be further enhanced using the several polymer layers to form multilayer PET. Oxygen scavengers may be incorporated in PET to reduce the level of oxygen in headspace and in the beverage and also reduce the oxygen ingress and increasing the shelf life (Bacigalupi et al. 2013).

Titanium dioxide (TiO_2) is often added to HDPE or PET containers to protect specialty drinks and milk against UV rays that are detrimental to their quality and also compromise recyclability. But it adds to the packaging costs. A novel oPTI process developed by Plastic Technologies Inc. results into a pure PET bottle that offers nearly 50% opacity due to the incorporation of foam without any additive addition and protects the products like drinkable yogurts, milk, and specialty milk products from the adverse effects of UV rays. The bottle is also recyclable and light weighted. Some other advancement in packaging materials includes:

1. Ready-to-use peel polymers which provide easy opening and resilience
2. Biodegradable Styrofoam derived from milk
3. Synthetic labelling adhesives meant for labelling of glass bottle

Recently, the emerging swing is to develop the new packaging materials with improved food-package-environment communication. Surface treatments for glass containers are necessary to improve the hydrolytic resistance of glass surface. In a study reported by Naknikham et al. (2014), glass was rinsed with many solutions like ammonium sulfate, alum, acetic acid, and citric acid, then cleaned and dried for

20 min at 110 °C. Glass treated with 5% alum showed the improved hydrolytic resistance.

Incorporation of nanoparticles in coating (Nanolok™ Technology) may enhance the oxygen barrier greater than four times than the PVDC-coated PET when RH varies from 0% to 80%. This coating will provide moisture barrier similar to PVDC-coated PET when examined at 85% RH and 40 °C. Such improvements in the barrier properties over a wide range of relative humidity are beneficial to enhance the flexible packaging performance to control the food quality and thus maintain the shelf life. R-Flute, the latest corrugated fluting, was developed by DS Smith Packaging that offers better surface for presentation and printing; Epotal Eco developed by BASF is the first of its kind compostable water-based adhesive that is certified by TÜV. Thus it offers the opportunities for developing multilayer films from biodegradable packaging materials which can be used as wrap for chocolate bar or potato chips.

Food Safety and Environmental Issues

The emerging innovations in packaging of food no doubt assure the quality of food products, but the safety of new techniques is still a great concern. This concern is due to the several problems especially faced during distribution of chilled food where a minute fluctuation in temperature may harm the food leading to spoilage. Nowadays temperature sensor embedded in RFID tags can be used along with their integration with physical and chemical sensors to provide traceability systems which is temperature maintained (Abad et al. 2009). The safety issues about the active and intelligent packaging may be considered because of the following factors:

1. The contents must be properly labelled to prevent the misuse by consumers.
2. The migration of active and intelligent compounds inside the food from package is also a big safety concern. Therefore, the active materials must be nontoxic in nature and comply with food regulations.
3. The working efficiency of active or intelligent packaging must preserve the food products without affecting their quality. They should provide reliable information about food spoilage or growth of microorganism in food matrices (Majid et al. 2016).

Surely nanotechnology has worked like a magical spell for food and beverage packaging industry; however its safety is still not sure. Nanoploymers when incorporated in suitable polymer may dramatically improve their characteristics. However, the additives with GRAS status must be evaluated once when employed at nanoscale to meet food safety regulations because the nanoparticles are more mobile, reactive, and toxic. Therefore the contents of nanoparticles must go through food safety assessment by suitable scientific association before their use in the beverage and food products as well as in packaging.

The environmental policies are necessary to meet the sustainable growth, but it puts extra cost during the food supply chain. Packaging materials are the main contributors to the solid waste stream. Therefore, the packaging industry is working hard to develop packaging materials with altered structure making them more eco-friendly and biodegradable (Bechini et al. 2008). One such material is polylactic acid which is composed of repeating lactic acid monomer; however it is still not widely used due to its high cost. Generally corn is employed as source material to prepare lactic acid required to be polymerized. Further soy based may be blended with polyester to invent novel packaging materials.

Future Trends

The manufacturers as well as processors must innovate the packaging more and more to retain the consumers worldwide. The food companies should make their customers brand loyal that is difficult to achieve due to the several unique choices consumer has. Digital printing can be seen as a good option for manufacturers to attach with consumers on a regional, individual, or emotional level. Active and intelligent packaging has no doubt several advantages than others, but scientists should also develop next-generation hybrid packaging providing environmental and functional benefits. The demand for biodegradable packages is on rise due to growing concern on sustainability which can be a strong purchase driver if price and quality for same product are similar. The packaging materials must be repurposable and reusable. There must be more versatility in pack sizes to meet the consumer requirements.

Conclusion

The rising consumers' demand for the natural, wholesome, and safe products has driven the packaging industry towards innovative packaging systems which can improve the safety and quality along with retaining the organoleptic attributes as well as the nutritional content of food and beverage and controlling the food-borne pathogens. Novel active/intelligent packaging materials are being developed to produce the sustainable, safe, and eco-friendly packaging solutions. However several safety issues such as degradation of the product quality and negative effects on human health must be resolved before their use. Further the twenty-first-century innovations are incomplete with nanotechnology which contributes greatly to improve the barrier and mechanical properties of container/package and design the amazing sensing technologies. The developments in biopolymer-based biodegradable packaging combining with nanotechnology result in reductions of wastage assuring the sustainability and also improve the package properties like never before.

Therefore, there must be complete understanding of working principles, mechanisms, and their optimal use to synthesize the packaging systems which would be efficient to maintain the food quality and safety. Currently, very less products are packed in new packaging systems in comparison to the number of research work carried till date. However, innovative technologies are expected to commercialize at a bigger platform in future. There is a strong need of collaboration of industry, prominent research institutions, and government agencies for the success of novel packaging techniques for food and beverage applications.

References

Abad E, Palacio F, Nuin M et al (2009) RFID smart tag for traceability and cold chain monitoring of foods: demonstration in an intercontinental fresh fish logistic chain. J Food Engg 93:394–399

Abdellah AM, Ken AI (2012) Effect of storage packaging on sunflower oil oxidative stability. Am J Food Technol 7:700–707

Ahvenainen R (2003) Active and intelligent packaging: an introduction. In: Ahvenainen R (ed) Novel food packaging techniques. Woodhead Publishing Ltd., Cambridge, UK, pp 5–21

Akbar A, Anal AK (2014) Zinc oxide nanoparticles loaded active packaging, a challenge study against *Salmonella typhimurium* and *Staphylococcus aureus* in ready-to-eat poultry meat. Food Control 38:88–95

Almenar E, Catala R, Hernandez-Munoz P, Gavara R (2009) Optimization of an active package for wild strawberries based on the release of 2-nonanone. LWT-Food Sci Technol 42:587–593

Anthierens T, Ragaert P, Verbrugghe S et al (2011) Use of endospore-forming bacteria as an active oxygen scavenger in plastic packagingmaterials. Innov Food Sci Emerg Technol 12(4):594–599

Bacigalupi C, Lemaistre MH, Boutroy N et al (2013) Changes in nutritional and sensory properties of orange juice packed in pet bottles: an experimental and modelling approach. Food Chem 141:3827–3836

Barbosa-Pereira L, Aurrekoetxea GP, Angulo I et al (2014) Development of new active packaging films coated with natural phenolic compounds to improve the oxidative stability of beef. Meat Sci 97(2):249–254

Basch C, Jagus R, Flores S (2013) Physical and antimicrobial properties of tapioca starch-HPMC edible films incorporated with nisin and/or potassium sorbate. Food Bioprocess Tech 6(9):2419–2428

Bechini A, Cimino M, Marcelloni F et al (2008) Patterns and technologies for enabling supply chain traceability through collaborative e-business. Inf Softw Technol 50:342–359

Blanco MM, Molina V, Sanchez M et al (2014) Active polymers containing *Lactobacillus curvatus* CRL705 bacteriocins: effectiveness assessment in Wieners. Int J Food Microbiol 178:7–12

Bodenhamer WT (2000) Method and apparatus for selective biological material detection. US patent 6, 051, 388 (Toxin Alert Inc. Canada)

Brockgreitens J, Abbas A (2016) Responsive food packaging: recent progress and technological prospects. Compr Rev Food Sci Food Saf 15:3–15

CAEN RFID (2017) CAEN RFID easy2log© RT0005. http://www.caenrfid.it/en/Caen Prod.jsp?mypage=3&parent=65&idmod=780 Accessed 14 May 2017

Calatayud M, López-de-Dicastillo C, López-Carballo G et al (2013) Active films based on cocoa extract with antioxidant, antimicrobial and biological applications. Food Chem 139:51–58

Camo J, Beltran JA, Roncales P (2008) Extension of the display life of lamb with an antioxidant active packaging. Meat Sci 80:1086–1091

Campos CA, Gerschenson LN, Flores SK (2011) Development of edible films and coatings with antimicrobial activity. Food Bioprocess Technol 4:849–875
Clariant (2017) Oxygen protection for packaged foods. http://www.clariant.com/oxy-guard-oxygen-scavenger Accessed 16 May 2017
Coma V (2008) Bioactive packaging technologies for extended shelf life of meat-based products. Meat Sci 78:90–103
CSL (2017) CS8304 cold chain temperature logging tag. http://www.convergence.com. hk/products/rfid/rfid-tags/cs8304/. Accessed 11 May 2017
Dainelli D, Gontard N, Spyropoulos D et al (2008) Active and intelligent food packaging: legal aspects and safety concerns. Trends Food Sci Technol 19:S103–S112 http:// dx.doi.org/10.1016/j.tifs.2008.09.011
Day BPF (2003) Active packaging. In: Coles R, McDowell D, Kirwan M (eds) Food packaging technologies. CRC Press, Boca Raton, pp 282–302
Day BPF (2008) Active packaging of food. In: Kerry J, Butler P (eds) Smart packaging technologies for fast moving consumer goods. Wiley, New York, pp 1–18
de Abreu PDA, Cruz JM, Losada PP (2012) Active and intelligent packaging for the food industry. Food Rev Int 28:146–187
de Abreu PDA, Losada PP, Maroto J et al (2011) Natural antioxidant active packaging film and its effect on lipid damage in frozen blue shark (*Prionace glauca*). Innov Food Sci Emerg Technol 12(1):50–55
De La Puerta MCCN, Gutierrez BC, Sanchez JC (2010) Smart packaging for detecting microorganisms. US Patent US8741596 B2, 21 Apr 2010
Doyle ME (2006) Nanotechnology: a brief literature review. Food Research Institute Briefings [Internet] https://fri.wisc.edu/files/Briefs_File/FRIBrief_Nanotech_Lit _Rev.pdf. Accessed 14 May 2017
EFSA (2014) Scientific opinion on the safety assessment of the active substances, palladium metal and hydrogen gas, for use in active food contact materials. EFSA J 12(2):3558–3566
El Amin A (2007) Nanoscale particles designed to block UV light. http://foodproductiondaily.com/news/ng.asp?id=80676 Accessed 18 May 2017
ETC Group (2004) ETC group report down on the farm: the impact of nano-scale technologies on food and agriculture http://www.nanowerk.com/nanotechnology/ reports/reportpdf/report10.pdf. Accessed 29 May 2017
Etienne M, Ifremer N (2005) SEAFOODplus-traceability-valid-methods for chemical quality assessment-Volatile amines as criteria for chemical quality assessment. http://archimer.ifremer.fr/doc/2005/rapport-6486.pdf. Accessed 13 May 2017
EU (2009) Guidance to the commission regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. Version 10. European Commission Health and Consumers Directorate- General Directorate E-Safety of the Food chain. E6- Innovation and sustainability
Ferrari MC, Carranzaa S, Bonnecazea RT et al (2009) Modeling of oxygen scavenging for improved barrier behavior: blend films. J Membr Sci 329:183–192
Ferrocinoa I, Greppia A, La Storiab A et al (2016) Impact of nisin-activated packaging on microbiota of beef burgers during storage. Appl Environ Microbiol 82:549–559
Freshpoint (2017a) BestBy. http://www.freshpoint-tti.com/time-from-opening-indicators/. Accessed 15 May 2017
Freshpoint (2017b) BestBy. http://www.freshpoint-tti.com/technology/. Accessed 15 May 2017
Georgescu I, Cobianu C, Dumitru VG (2008) Intelligent packaging method and system based on acoustic wave devices. US patent US 7755489 B2, 28 Apr 2008
Gontard N (2000) Panorama des emballages alimentaire actif (Panorama of active food packaging). In: Gontard N (ed) Les Emballages Actifs. Tech & Doc Editions, Londres. ISBN-10: 2743003871
Gunders D (2012) Wasted: how America is losing up to 40 percent of its food from farm to fork to landfill. NDRC Issue Paper IP:12–06-B https://www.nrdc.org/sites/default /files/wasted-food-IP.pdf. Accessed 6 May 2017

Hong SI, Park WS (2000) Use of color indicators as an active packaging system for evaluating kimchi fermentation. J Food Eng 46:67–72

Insignia Technologies (2017) Novas: embedded label. http://insignia.mtcserver11.com/ portfolio-view/novas-embedded-label/. Accessed 3 May 2017

Jamshidian M, Tehrany EA, Imran M et al (2012) Structural, mechanical and barrier properties of active PLA-antioxidant films. J Food Eng 110(3):380–389

Jayasena DD, Jo C (2013) Essential oils as potential antimicrobial agents in meat and meat products: a review. Trends Food Sci Technol 34:96–108

Jin T, Zhang H (2008) Biodegradable polylactic acid polymer with nisin for use in antimicrobial food packaging. J Food Sci 73:127–134

Jofré A, Aymerich T, Garriga M (2008) Assessment of the effectiveness of antimicrobial packaging combined with high pressure to control *Salmonella* sp. in cooked ham. Food Control 19(6):634–638

Johns Hopkins University Applied Physics Laboratory (2014) A colorimetric sensor of food spoilage based on a molecularly imprinted polymer. http://www.jhuapl.edu/ott/technologies/technology/articles/P01491.asp. Accessed 18 May 2017

Joseph T, Morrison M (2006) Nanotechnology in agriculture and food. A nanoforum report https://cordis.europa.eu/pub/nanotechnology/docs/nanotechnology_in_agriculture_and_food.pdf. Accessed 22 May 2016

Kang HJ, Jo C, Kwon JH et al (2007a) Effect of pectin-based edible coating containing green tea powder on the quality of irradiated pork patty. Food Control 18(5):430–435

Kang S, Pinault M, Pfefferle LD et al (2007b) Single-walled carbon nanotubes exhibit strong antimicrobial activity. Langmuir 23:8670–8673

Keep-it Technologies (2017) The shelf life indicator. http://keep-it.com/Accessed 2 May 2017

Kerry JP (2014) New packaging technologies, materials and formats for fast-moving consumer products. In: Han JH (ed) Innovations in food packaging, 2nd edn. Academic, San Diego, pp 549–584

Kerry JP, O'Grady MN, Hogan SA (2006) Past, current and potential utilisation of active and intelligent packaging systems for meat and muscle-based products: a review. Meat Sci 74:113–130

Knee M (1990) Ethylene effects in controlled atmosphere storage of horticultural crops. In: Calderon M, Barkai-Golan R (eds) Food preservation by modified atmospheres. CRC Press, Boca Raton, pp 225–235

Laboratories STANDA (2017a) ATCO®. http://www.standa-fr.com/eng/laboratoiresstanda/ atco/. Accessed 4 May 2017

Laboratories STANDA (2017b) SANICO® is our range of antifungal coatings for the agro-food industry. http://www.standa-fr.com/eng/laboratoires-standa/sanico/Accessed 4 May 2017

Lagaron JM (2005) Bioactive packaging: a novel route to generate healthier foods. Paper presented at 2nd conference in food packaging interactions, Campdem (CCFRA), Chipping Campden, UK, 14–15 Jul 2005

Landau S (2007) The future of flavor and odor release. Paper presented at Intertech Pira conference on the future of caps and closures-latest innovations and new applications for caps and closures, Atlanta, 20–21 June 2007

Lawrie K, Mills A, Hazafy D (2013) Simple inkjet-printed, UV-activated oxygen indicator. Sens Actuators B Chem 176:1154–1159

Lee DS (2014) Antioxidant packaging system. In: Han JH (ed) Innovations in food packaging. Academic, San Diego, pp 111–131

Lee DS, Yam KL, Piergiovanni L (2008) Food packaging science and technology. CRC Press, New York, pp 243–274

LINPAC (2012) LINPAC packaging partners Addmaster to tackle packaging bugs. http://www.linpacpackaging.com/pt-pt/news/201208/linpac-packaging-partnersadd master-tackle-packaging-bugs. Accessed 21 May 2017

LINPAC (2017) Not just trays and film. https://www.linpacpackaging.com/en/not-just-trays-and-film. Accessed 15 May 2017

Liu XH, Xie SY, Zhou LB et al (2013) Preparation method of nano TiO_2 powder and method for preparing oxygen gas indicator from nano TiO_2 powder. China patent CN103641163A, 28 Nov 2013

Lloret E, Picouet P, Fernández A (2012) Matrix effects on the antimicrobial capacity of silver based nanocomposite absorbing materials. LWT Food Sci Technol 49:333–338

Lopez-Rubio A, Gavara R, Lagaron JM (2006) Bioactive packaging: turning foods into healthier foods through biomaterials. Trends Food Sci Technol 17:567–575

Lövenklev M, Artin I, Hagberg O et al (2004) Quantitative interaction effects of carbon dioxide, sodium chloride, and sodium nitrite on neurotoxin gene expression in nonproteolytic *Clostridium botulinum* type B. Appl Environ Microbiol 70:2928–2934

Majid I, Nayik GA, Dar SM et al (2016) Novel food packaging technologies: innovations and future prospective. J Saudi Soc Agric Sci https://doi.org/10.1016/j.jssas. 2016.11.003

Marcos B, Aymerich T, Monfort JM et al (2007) Use of antimicrobial biodegradable packaging to control *Listeria monocytogenes* during storage of cooked ham. Int J Food Microbiol 120:152–158

Marcos B, Aymerich T, Monfort JM et al (2008) High-pressure processing and antimicrobial biodegradable packaging to control *Listeria monocytogenes* during storage of cooked ham. Food Microbiol 25:177–182

Martínez-Olmos A, Fernández-Salmerón J, Lopez-Ruiz N et al (2013) Screen printed flexible radiofrequency identification tag for oxygen monitoring. Anal Chem 85:11098–11105

Maxwell Chase Technologies (2017) Fresh-R-Pax® trays. http://www.maxwellchase.com/food-packaging/absorbent-trays/. Accessed 4 May 2017

McAirlaid (2017) MeatPad. http://www.meatpads.info/en/Accessed 25 May 2017

Mennecke B, Townsend A (2005) Radio frequency identification tagging as a mechanism of creating a viable producer's brand in the cattle industry. Midwest Agribusiness Trade Research and Information Center, Iowa State University, Ames USA http://www.card.iastate.edu/products/publications/pdf/05mrp8.pdf. Accessed 1May 2017

Miller G, Senjen R (2008) Out of the laboratory and on to our plates – Nanotechnology in food and agriculture. http://libcloud.s3.amazonaws.com/93/b5/4/547/ Nanotechnology_in_food_and_agriculture_-_web_resolution.pdf. Accessed 7 May 2017

Mitsubishi Gas Chemical (2017a) AGELESS® http://www.mgc.co.jp/eng/products/abc/ageless/index.html. Accessed 15 May 2017

Mitsubishi Gas Chemical (2017b) AGELESS OMAC® oxygen absorbing film. http://ageless.mgc-a.com/product/ageless-omac/. Accessed 15 May 2017

NORDENIA (2011) Nor®Absorbit makes your food nice and crispy. http://www.worldpressonline.com/PressRelease/nordenia-innovative-packaging-for-microwave-dishes-31607.html. Accessed 12 May 2017

Naknikham U, Jitwatcharakomol T, Tapasa K et al (2014) The simple method for increasing chemical stability of glass bottles. Key Eng Mater 608:307–310

O'Grady MN, Kerry JP (2008) Smart packaging technology. In: Toldra F (ed) Meat biotechnology. Springer, New York, pp 425–451

Ozdemir M, Floros JD (2004) Active food packaging technologies. Crit Rev Food Sci Nutr 44:185–193. https://doi.org/10.1080/10408690490441578

Perry MR, Lentz RR (2009) Susceptors in microwave packaging. In: Lorence MW, Pesheck PS (eds) Development of packaging and products for use in microwave ovens. Woodhead Publishing Limited Cambridge, UK, pp 207–236

Pospiskova K, Safarik I, Sebela M et al (2012) Magnetic particles-based biosensor for biogenic amines using an optical oxygen sensor as a transducer. Microchim Acta 180:311–318

Realini CE, Marcos B (2014) Active and intelligent packaging systems for a modern society. Meat Sci 98(3):404–419

Regier M (2014) Microwavable food packaging. In: Han JH (ed) Innovations in food packaging, 2nd edn. Academic, San Diego, pp 495–514

Regier M, Knoerzer K, Schubert H (2016) The microwave processing of foods, 2nd edn. Woodhead Publishing Ltd, Cambridge, pp 273–299

Rooney ML (1995) Overview of active packaging. In: Rooney ML (ed) Active food packaging. Blackie Academic and Professional, Glasgow, pp 1–37
Salinas Y, Ros-Lis JV, Vivancos JL et al (2014) A novel colorimetric sensor array for monitoring fresh pork sausages spoilage. Food Control 35:166–176
Sealed Air (2017) Cryovac® OS films-rapid headspace. http://www.cryovac.com/NA/EN/pdf/osfilms.pdf. Accessed 10 May 2017
SEALPAC (2014) TenderPac-best meat quality, appetizing appearance. http://www.sealpac.de/fileadmin/user_upload/media/innovations/verpackungsloesungen/TenderPac_2014_online-EN.pdf. Accessed 17 May 2017
Sen L, Hyun KH, Kim JW et al (2013) The design of smart RFID system with gas sensor for meat freshness monitoring. Adv Sci Technol Lett 41:17–20
Sirane (2011) A-Crisp™ boxes, boards, sleeves and liners for crisping in a microwave. http://www.sirane.com/microwave-susceptors-crisp-it-range/sira-cook-crisp-it-susceptor-boards-boxes.html. Accessed 26 May 2017
Smith AJ, Poulston S, Rowsell L et al (2009) A new palladium-based ethylene scavenger to control ethylene-induced ripening of climacteric fruit. Platin Met Rev 53:112–122
Smolander M (2000) Freshness indicators for direct quality evaluation of packaged foods. Paper presented at International conference on active and intelligent packaging, Chipping Campden, UK 7–8 Sept 2000 pp 1–16
Sozer N, Kokini JL (2009) Nanotechnology and its applications in the food sector. Trends Biotechnol 27:82–89
Suppakul P, Miltz J, Sonneveld K et al (2003) Active packaging technologies with an emphasis on antimicrobial concise reviews in food science. J Food Sci 68:408–420
Tempra Technology™ (2017) Self chilling cans, Tempra Technology™ Florida, USA. http://www.tempratech.com/portfolio/i-c-cans/. Accessed 23 May 2017
Timestrip (2016) Timestrip® cold chain products for food. http://timestrip.com/products/food-range/. Accessed 15 May 2017
Uniform Code Council (2017) GS1 databar family. Available from: Lawrenceville NJ: Uniform Code Council https://www.gs1.org/barcodes/databar. Accessed 6 June 2017
Vermeiren L, Heirlings L, Devlieghere F et al (2003) Oxygen, ethylene and other scavengers. In: Ahvenainen R (ed) Novel food packaging techniques. CRC Press, USA, pp 5–49
VITSAB (2015) Seafood TTI labels. http://vitsab.com/?page_id=1983. Accessed 2 May 2017
Wanihsuksombat C, Hongtrakul V, Suppakul P (2010) Development and characterization of a prototype of a lactic acid–based time–temperature indicator for monitoring food product quality. J Food Engg 100:427–434
Yam KL, Takhistov PT, Miltz J (2005) Intelligent packaging: concepts and applications. J Food Sci 70:R1R10
Yeh JT, Cui L, Chang CJ et al (2008) Investigation of the oxygen depletion properties of novel oxygen-scavenging plastics. J Appl Polym Sci 110:1420–1434
Yoshida CMP, Maciel VBV, Mendonça MED (2014) Chitosan bio-based and intelligent films: Monitoring pH variations. Food Sci Technol-LEB 55:83–89
Zagory D (1995) Ethylene-removing packaging. In: Rooney ML (ed) Active food packaging. Blackie Academic and Professional, Glasgow, pp 38–54
Zelzer M, Todd SJ, Hirst AR et al (2013) Enzyme responsive materials: design strategies and future developments. Biomater Sci 1:11–39
Zenner BD, Benedict CS (2002) Polymer compositions containing oxygen scavenging compounds. US Patent, 6391406, 21 May 2002
Zhai RC (2010) Intelligent packaging bottle with voice advertisement. China patent CN201784843U, 22 Mar 2010

Chapter 14
Role of Consumers in Innovation of Novel Food and Beverages

Shalini Sehgal

Abstract Innovation is the key driver of economic growth through the generation, dissemination, and use of knowledge. Thus, the success of any industrial sector relies on the extent of innovation. In the food industry, new food product development is highly dependent on the consumer perception and acceptance, and therefore it is of utmost importance to include the consumer in the development process. This helps in lowering down the probability of product failure in the market. The market studies and sensory analyses are the most commonly used tools. In the food industry, like any other industry, product and process development is considered a vital part of a successful business strategy. The sectors of the food industry where important developments and innovation are registered include the processing technologies and the packaging systems, where the latest progresses have produced very significant outcomes.

Keywords Innovation · Novel · Functional · Consumer · Food industry

Introduction

Innovation is the process of transforming a discovery (i.e., idea, invention) into a good(s) or service(s) that consumers/customers are willing to purchase (Sam Saguy 2011). It has multiple facets such as science, technology, marketing and organization, partnership, risk, and social responsibility. Discoveries must be translated into products, services, or processes that must reach out and finally be the part of the economy for the society. Any idea/invention which can be replicated at an economical cost and satisfies a specific need is an innovation. The ideas that are suitable for implementation lead to innovation. Product innovation could be a major success factor in today's aggressive and competitive food markets (Suwannaporn and Speece 2010).

S. Sehgal (✉)
Department of Food Technology, Bhaskaracharya College of Applied Sciences, University of Delhi, Dwarka, New Delhi, India
e-mail: shalini.sehgal@bcas.du.ac.in

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_14

The main objective while developing a new food product is to ensure that it will be accepted by consumers as this acceptance is critical as it is based on an intimate relationship between the certain attributes of the product and the consumer perception and psychological response (Guine et al. 2012).

Role of Consumer

The role of the consumer is essential in the innovation processes in the food industry. Consumer demands in the field of food have changed drastically in the last decade. They have growing concerns with regard to the safety and quality of food supply chain. These have influenced and will continue to influence their perceptions of emerging novel food processes. The factors influencing consumer acceptance of novel foods and beverages can be generally grouped as follows:

1. Consumer involvement
2. Food neophobia
3. Perceived benefits and risks of the new food products
4. Concerns pertaining to the long-term effects of novel technologies on human health
5. Threat to the food chain and the environment by the new technology/process
6. Cultural, psychosocial, and lifestyle factors of the consumers

Emergence of new technologies has the potential to deliver some tangible benefits to consumers. These can include extended shelf life and improved nutritional and sensory profiles among others such as ready to eat, ease of convenience of preparation, and better packaging designs. But on the other hand, these emerging technologies also raise concerns among consumers, for example, food irradiation has been not accepted in totality till date.

In 2007, Beckeman and Skjoldebrand stated that innovation in the food industry combines technological innovation with social and cultural innovation. But, the degree of innovation is still low in the food industry. The radical or really new innovations are not often introduced in the food market, but yet a number of new technologies are already available or being further investigated at present by various workers.

Innovation in the food sector faces is very challenging. One such challenge is food neophobia, which is the fear of new foods. Although, this phenomenon usually pertains to children, but for some adults, food neophobia continues in adulthood too. Such individuals form one dimension of the overall consumer population which cannot be neglected during the new product development process and marketing studies (Guine et al. 2013; Henriques et al. 2009). The neophobic consumers must not be neglected by consumer research and marketing team who usually focus on those who are interested in new products only.

Consumers' perceptions of novel processes across different technologies have revealed that the greatest consumer concern was for the most radical innovations. These include mainly genetic modification and food irradiation. They are reluctant to accept genetically modified and /or irradiated foods due to the fact that they are very much averse to the associated risks (Chen et al. 2013; Costa Font et al. 2008).

The assessment of consumer perceptions toward foods is of paramount importance in the development and marketing of products (da Silva et al. 2014a, b). The development of effective food marketing and communication strategies depends on the understanding on how consumers respond to information (Verbeke and Liu 2014). Communication and information can shape the attitudes of consumers and influence their choices and behavior without changing the attributes of the novel products.

Concept of Open Innovation

Human capital inputs of innovation processes, i.e., the individual skills and knowledge employed in research and development and commercialization activities, can be sourced from both inside and outside the corporate boundaries (Sarkar and Costa 2008). They are categorized into two broad categories. Closed innovation (CI) processes are sourced mainly within an organization's boundaries. On the other hand, open innovation (OI) processes have inputs, which are to a large extent, sourced from outside the firm.

Chesbrough (2003) defined OI as "valuable ideas can come from inside or outside the company and go to market from inside or outside the company as well" and later redefined the concept in 2006 as "the use of purposive inflows and outflows of knowledge to accelerate internal innovation, and expand the markets for external use of innovation, respectively".

OI is not a new concept for the food industry. Manceau et al. (2011) have shown that the food industry has been collaborating with suppliers, consumers, customers, and academia on a variety of topics, for example, new product development (NPD), sensory evaluation, consumer research, ingredients, and their functionality.

Collaborative Product Innovation

Consumers should be involved as a part of the innovation process as it improves the probability of successful innovation as majority of the new food and beverage products eventually fail on commercialization. The connection between the consumer and the product is essential.

Kemp (2013) has stated that the consumer-driven food and beverage innovations should be based on the consumer needs only. This can be achieved through a company culture focused on the consumer input. Appropriate consumer input throughout the innovation process is important. This collaboration between the consumer and the company is named collaborative product innovation (CPI). CPI is also termed as co-creation or co-innovation. It offers a new way to innovate, in which consumers work in collaboration with companies to develop products which are mutually beneficial to both the parties. This helps in improving the hypothesis of new product success. At present, often, this collaboration is achieved by means of social networks.

The main focus is on consumer in the field of food, but determinants of consumer adoption of innovations can be studied from different angles and perspectives in various disciplines as stated by Ronteltap et al. (2007).

Cooperation and integration of existing knowledge from different organizations are achieved across food supply chains under co-innovation. Estrada-Flores (2010) discusses the advantages of using a cohesive approach to food co-innovation. The study discusses the approach to recognize the highly dynamic nature of the food manufacturing industry including the benefits of market-driven innovation. It also emphasizes the usefulness of policy as an instrument to encourage innovation. The stated sustainable co-innovation framework can create a paradigm shift in the way food chain participants innovate.

CPI is highly suitable to tap customers for innovative concepts, support technology development, and create value networks for new product launch. Costa (2013) refers that collaborating with expert technology users can promote value addition to companies by generating new product ideas and supporting internal research and development. Furthermore, CPI can accelerate the adoption of novel foods by increasing technology acceptance in selected market segments.

Consumer Perception and Sensory Analysis

Consumer food behavior depends on two classes of variables: behavioral and attitudinal (Cardello et al. 2000). Behavioral variables include measures like preference, buying ability, and pattern of consumption, whereas attitudinal involves the assessment of like/dislike, pleasantness/unpleasantness, or measures of the desire to select or eat foods.

Sensory method of evaluation is used to measure, analyze, and interpret those responses to foods as perceived through the senses of sight, smell, touch, taste, and hearing. It is a science-based method. Guine et al. (2010) also stated that the sensory parameters such as appearance, odor, flavor, and texture are also important attributes, and these contribute to the quality of food products.

Moskowitz and Hartmann (2008) have emphasized that the consumer role is very critical for innovation in the food industry. Therefore, developing a successful new product requires a correct sensory evaluation and a complete understanding of the consumers' acceptance criteria (Guine 2012).

The focus on consumer has resulted in the sensory analysis being one of the important stages for new product development. In fact, for the success of one type of product in the market, it is very important to direct it to the right consumer segment. Food producers must know market orientation and understand the consumer needs and expectations.

Consumers' current and future needs as well as its determinants aid in the development of new products as reported by Guine in 2012.

Innovation in the Food Industry

The food industry faces a huge number of challenges such as changing consumer needs, shortened product life cycles, competitive time-to-market race, cluttered retail shelf space, and increasing difficulty in meeting the heterogeneous requirements of a growing number of supply chain factors such as suppliers, customers, or regulators (Bellairs 2010).

The food industry is traditionally regarded as a sector with low research intensity as compared to other sectors but the innovation has become of great interest in this sector also. Innovation helps the food companies to stand out from their competitors and also generate consumer interest (Bigliardi and Galati 2013).

Many studies have revealed that there has been consumer acceptance of technology-based innovations in food sector. But the acceptance of some has been easier and some have been totally rejected as reported by Cardello (2003). Noticeable examples of generally rejected innovations are genetically modified foods (GMFs) in Europe and food irradiation (Ronteltap et al. 2007). Consumer acceptance of technology-based innovations depends on their ability of usage. The determinants of acceptance are classified as follows:

Determinants of acceptance	
Proximal	Distal
Perceived costs and benefits, like usage and health-related benefits	Innovation features, e.g., price, taste properties, and convenience
Perceived risks and uncertainty, such as safety issues, consumer concerns, emotions, and trust	Consumer characteristics, e.g., sociodemographics, knowledge, personality, general attitudes, and values
Subjective norms, e.g., social and peer pressure	The social system, e.g., the economic, political, and social environment
Perceived behavioral control, like self-efficacy	

The developments in the food industry have taken place in various related fields. However, there are three areas where the innovation has been particularly important in the past decade: food processing, food packaging, and new trends toward developing healthier foods.

Innovative Processing Technologies

There has been an ever increasing demand for more convenient and varied food products in the last decade. The emphasis is on faster and more efficient production techniques, improved quality, and enhanced safety and longer shelf life. Emergence of novel processing technologies such as thermal ones which include radio frequency and ohmic heating highlights this trend. On the other hand, nonthermal processing technologies such as pulsed electric fields, high hydrostatic pressure, pulsed light, and ultrasounds are being explored. Some of these techniques have developed considerably in the past years and are now being commercialized. In fact, some of them are being used in many food processing facilities around the world (Guine 2013).

Innovative Packaging

The developments, in the field of food packaging, are mainly aimed at extending shelf life, maximizing product quality, and pleasing the consumer in the last decade. Important innovations in the area of food packaging such as the edible films and coatings, the active and intelligent packaging, and the nanopolymers have been developed.

New Trends Toward Developing Healthier Foods

In developed countries, typical diets have been evolving toward highly caloric foods, rich in saturated fats and sugars, which have been associated with a number of chronic diseases. These diets also contain a very low content of complex carbohydrates and dietetic fiber, thus contributing to an increased disease risk. Furthermore, the problem is intensified by the lack of physical exercise. Health and food are on top priorities for people all across the globe (Smith and Charter 2010).

Dewapriya and Kim (2014) have stated that the functional foods and nutraceuticals are a means to improve nutrient intake. The ingestion of compounds with bioactive effects is beneficial for the human health and has been given considerable attention in the past decade.

Therefore, the market growth for functional foods presently overtakes that of the more traditional food products. Although the health characteristics for all types of food should be taken into account yet the current trend emphasizes more so, on the functional foods. (Smith and Charter 2011; Francieli da Silva et al. 2015).

Development of functional food products not only includes the incorporation of certain compounds (or ingredients) with established health benefits but also balancing of the sensory attributes such as taste, texture, and flavor. Yet apart from these, convenience remains crucial factors for consumers certainly influence their buying choice. The functional food and nutraceutical industries represent the most dynamic segment currently in the food sector. This segment is evolving toward a more research-oriented patterns, similar to pharmaceutical industries (Schieber 2012).

Conclusion

Consumers are becoming more demanding when it comes to food in term of its variety, safety, and quality. There has been an urgent need of innovations in the current scenario of globalization across the industrial sectors. Food sector has also been influenced by the concept of innovation. Although the innovation in food cannot be something very radical, the majority of innovations are consumer centric as the acceptance of the concept is critical to the product, process, or technological success. The success of functional foods and nutritional supplements has been due to their potential in convincing the consumers about their health benefits. In many countries, the functional food market seems to be dominated by gut health products, in particular probiotic products. A number of consumer studies have shown that consumers have poor knowledge and awareness levels along with high levels of skepticism toward the most radical food process innovations such as genetic modification and food irradiation. More so, foods produced by these novel processes have generally been perceived as unsafe, unwholesome, and unnatural by consumers. Consequently, given that food production methods have become an increasing cause of concern, a greater proportion of consumers are increasingly seeking "clean-labelled" minimally processed food products such as artisan foods and organic foods.

References

Beckeman M, Skjöldebrand C (2007) Clusters/networks promote food innovations. J Food Eng 79(4):1418–1425

Bellairs J (2010) Open innovation gaining momentum in the food industry. Cereal Foods World 55(1):4–6

Bigliardi B, Galati F (2013) Models of adoption of open innovation within the food industry. Trends Food Sci Technol 30(1):16–26

Cardello AV (2003) Consumer concerns and expectations about novel food processing technologies: effects on product liking. Appetite 40(3):217–233

Cardello AV, Schutz H, Snow C, Lesher L (2000) Predictors of food acceptance, consumption and satisfaction in specific eating situations. Food Qual Prefer 11(3):201–216

Chen XP, Li W, Xiao XF, Zhang LL, Liu CX (2013) Phytochemical and pharmacological studies on Radix Angelica sinensis. Chin J Nat Med 11(6):577–587

Chesbrough HW (2003) Open innovation: the new imperative for creating and profiting from technology, 1st edn. Harvard Business Review Press, Boston

Costa AIA (2013) Collaborative product innovation in the food service industry. Do too many cooks really spoil the broth? Open Innovation in the Food and Beverage Industry, 154–173

Costa Font M, Gil JM, Traill WB (2008) Consumer acceptance, valuation of and attitudes towards genetically modified food: review and implications for food policy. Food Policy 33(2):99–111

da Silva VM, Minim VPR, Ferreira MAM, de Paula Souza PH, da Silva Moraes LE, Minim LA (2014a) Study of the perception of consumers in relation to different ice cream concepts. Food Qual Prefer 36:161–168

da Silva GF, Rocha LW, Quintão N L M (2014b) Nutraceuticals, dietary supplements, and functional foods as alternatives for the relief of neuropathic pain. Bioactive nutraceuticals and dietary supplements in neurological and brain disease: prevention and therapy, p 87

Dewapriya P, Kim SK (2014) Marine microorganisms: an emerging avenue in modern nutraceuticals and functional foods. Food Res Int 56:115–125

Estrada-Flores S (2010) 'Understanding innovation in food chains', in C Mena & G Stevens (eds), Delivering performance in food Supply chains, Woodhead Publishing, Cambridge, UK, pp. 84–116

Francieli da Silva G, Rocha LW and Quintão NLM (2015) Chapter 10 - Nutraceuticals, Dietary Supplements, and Functional Foods as Alternatives for the Relief of Neuropathic Pain A2 -Watson, Ronald Ross. In: Preedy VR, editor. Bioactive Nutraceuticals and Dietary Supplements in Neurological and Brain Disease, San Diego: Academic Press; 2015, p. 87–93

Guine RP (2012) Sweet samosas: a new food product in the Portuguese market. Acad Res Int 2(3):70

Guine R (2013) Unit operations for the food industry: thermal processing & nonconventional technologies

Guine R, Lima MJ, Pato L, Correia AC, Gonçalves F, Costa E, Santos S (2010) Consumer study and sensorial evaluation of a newly developed spicy strawberry syrup. Int J Acad Res 2(3):173–178

Guine RP, Dias A, Peixoto A, Matos M, Gonzaga M, Silva M (2012) Application of molecular gastronomy principles to the development of a powdered olive oil and market study aiming at its commercialization. Int J Gastro Food Sci 1(2):101–106

Guine RP, Barros A, Queirós A, Pina A, Vale A, Ramoa H, Carneiro R (2013) Development of a solid vinaigrette and product testing. J Cul Sci Technol 11(3):259–274

Henriques AS, King SC, Meiselman HL (2009) Consumer segmentation based on food neophobia and its application to product development. Food Qual Prefer 20(2):83–91

Kemp SE (2013) Consumers as part of food and beverage industry innovation. Open innovation in the food and beverage industry. pp 109–138

Manceau D, Moatti V, Fabbri J, Kaltenbach PF, Bagger-Hansen L (2011) Open innovation – what is behind the buzzword. ESCP Europe and Accenture

Mena C, Stevens G (2010) Delivering performance in food supply chains. Cambridge (UK): Woodhead. p. 416–431. (Woodhead publishing series in food science, technology and nutrition; 185).

Moskowitz H, Hartmann J (2008) Consumer research: creating a solid base for innovative strategies. Trends Food Sci Technol 19(11):581–589

Ronteltap A, Van Trijp JCM, Renes RJ, Frewer LJ (2007) Consumer acceptance of technology-based food innovations: lessons for the future of nutrigenomics. Appetite 49(1):1–17

Saguy IS (2011) Paradigm shifts in academia and the food industry required to meet innovation challenges. Trends Food Sci Technol 22(9):467–475

Sarkar S, Costa AI (2008) Dynamics of open innovation in the food industry. Trends Food Sci Technol 19(11):574–580

Schieber A (2012) Functional foods and nutraceuticals. Food Res Int 46(2):437–572

Smith J, Charter E (2010) Functional food product development. Hoboken, NJ, USA: Wiley Blackwell Publishing

Suwannaporn P, Speece MW (2010) Assessing new product development success factors in the Thai food industry. British Food J 112(4):364–386

Verbeke W, Liu R (2014) The impacts of information about the risks and benefits of pork consumption on Chinese consumers' perceptions towards, and intention to eat, pork. Meat Sci 98(4):766–772

Chapter 15
IPRs in Respect to Food and Beverages

Sripathi Rao Kulkarni

Abstract The present chapter discusses the intellectual property (IP)-related issues with examples in the food and beverages industry. They form unseen backbone for the standing of a business entity in the market place. In today's competitive scenario, the strength in IP protection, enforcement, and monetization determines the strength of the business entity against its competitors. In view of dynamic consumer needs, many companies now are focusing on significant investments toward research and development and quick return on investments. This is applicable even to the food and beverages sector. There are several instruments for protection of IP rights such as patents, trademarks, copyrights, industrial designs, etc. All these factors enable the food and beverages sector lucrative and sought after.

Keywords Intellectual property rights · Foods · Beverages · Patents · Copyright · Trademarks · Industrial designs · Trade secrets · Geographical indications

Introduction

Energy, the significant driver for growth and mobility on our planet, can broadly be attributed to the quality and quantity of intake of food and beverages. The availability of food and beverages has become a point of concern with demographic explosion, changing climate, depletion in the natural flora and fauna, etc. To address such pertinent issues, multitudes of companies and organizations across the globe are focusing on ample supply of good quality food and nutrition to the people. In such pursuits, the entities explore the possibility of marked presence in the business by way of closely guarded IP instruments such as patents, trademarks, industrial designs, etc. with expansive territorial coverage and enforcement strategies toward significant profit-making. Those involved in the business of food and beverages mainly focus on protection of exclusivity rights pertaining to formulation,

S. R. Kulkarni (✉)
Business Development-Intellectual Property Unit, CSIR-Central Drug Research Institute, Lucknow, Uttar Pradesh, India
e-mail: sripathi_kulkarni@cdri.res.in

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_15

processing, packaging, and distribution of raw or finished products toward significant return on investments, as obtained exclusivities are for a limited period. The established business houses do negotiate the demands and timelines by managing financial commitments from other resources, whereas the micro, small, and medium establishments have to be careful in meeting the timelines toward successful deployment of the product into the market. The latter might receive serious threats if the IP instruments are not worked properly or unable to effectively negotiate the licensing arrangements or issues relating to transfer of technologies.

Food and Beverages Industry and IP Protection

A closer look into the food and beverages industry suggests that the food products would be fresh foods, such as prepared food as well as packaged foods including various alcoholic and nonalcoholic beverages (https://globaledge.msu.edu/industries/food-and-beverage/memo (as accessed on 30-03-2017)).

Foods/processed foods can be broadly classified into the following categories (https://www.foodsafety.gov/keep/types/ (as accessed on 30-03-2017))

(a) Meat
(b) Turkey
(c) Chicken and other poultry
(d) Seafood
(e) Eggs and egg products
(f) Dairy products
(g) Fruits and vegetable including juices
(h) Cereals, pulses, and nuts
(i) Baby food and infant formula
(j) Pet food

Types of beverages (https://en.wikipedia.org/wiki/Drink (as accessed on 30-03-2017))

(a) Milk
(b) Tea
(c) Coffee
(d) Carbonated drinks
(e) Fruit juices
(f) Alcoholic beverages

Constantly evolving consumer needs have resulted in a paradigm shift in the food and beverages industry. Strong R&D and innovation in the existing products is paramount to keep pace with changing customer trends and effective marketing strategies. Hence intellectual property rights play a pivotal role in safeguarding the company's innovative products and ideas in order to increase their commercial gains and to avoid conflict of interest (http://www.fooddive.com/news/top-10-food-and-beverage-industry-trends-and-why-they-matter/404484/ (as accessed on 30-03-2017)).

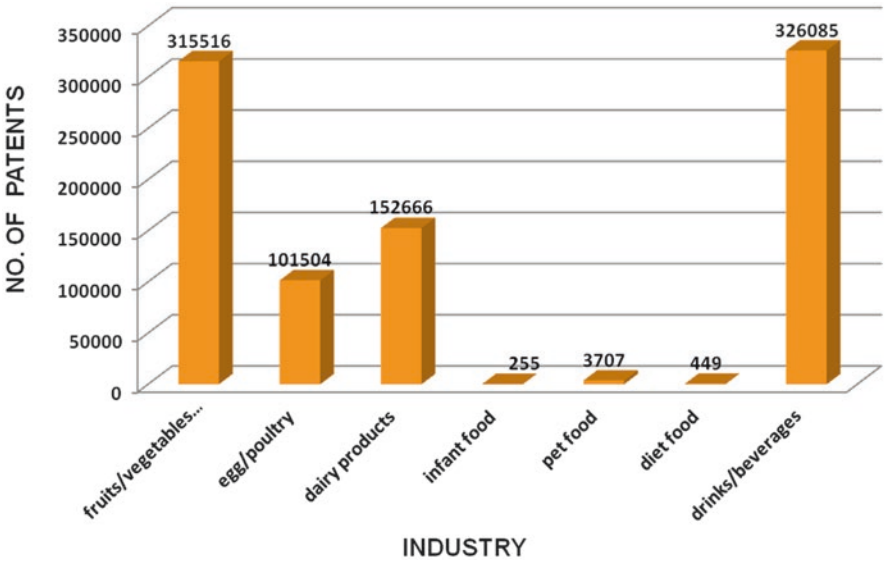


Fig. 15.1 Number of patent applications in various categories

Types of IPR protection in food and beverages industry

1. Patents
2. Copyrights
3. Trademarks
4. Industrial designs
5. Trade secrets
6. Geographical indications

Patents

According to the definition provided by World Intellectual Property Organization (WIPO), "a patent is an exclusive right granted for an invention, which is a product or a process that provides, in general, a new way of doing something, or offers a new technical solution to a problem" (http://www.wipo.int/patents/en/).

Patents in food industry cover almost everything from composition to processing, packaging, and finally marketing of the final product. An inventor can patent a process, a machine, an apparatus, or a composition of matter. A new food can be patented as a composition of matter. Figure 15.1 indicates the number of patent applications in various categories of food both raw and processed.

New fruit juices and beverage recipes can also be protected by way of filing patents. There has been a substantial growth in the segment of fruit juice market in India due to an increased awareness to health and fitness along with intelligent

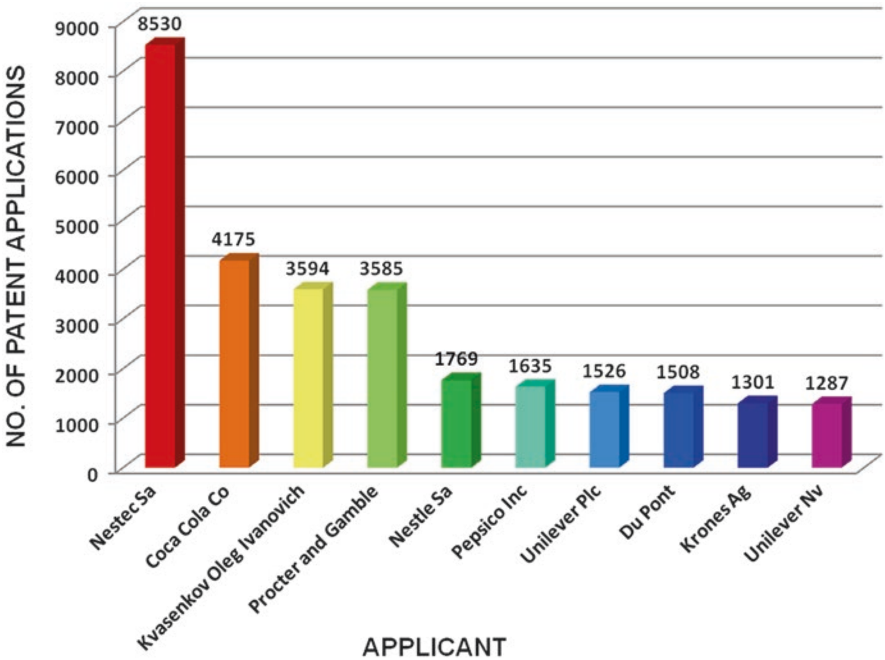


Fig. 15.2 Patent applicants in the beverage industry

market strategies. This has finally led to increased patent filings in this sector with the aim to capture a larger market share.

The trend of patent filling in Indian patent office gives a glimpse of the major key players in this segment. The organizations and institutes which have maximum intellectual property protection in this segment are Coca-Cola Company, Hindustan Unilever Limited, and Jagdale Industries Limited. Among the research institutes, Council of Scientific and Industrial Research (CSIR), Indian Institute of Technology (IIT), and Thapar Institute of Engineering and Technology have the highest number of intellectual property protection under this segment and seem to be doing substantial research in this field. Figure 15.2 represents the major patent applicants in the beverage industry.

Case Studies Pertaining to Food and Beverages Industry

Quorn: Meat Substitute

Increasing population and the effect of cattle rearing for meat have motivated the researchers to find alternative to meat per se. A breakthrough in this effort was achieved by Marlow Foods currently owned by Monde Nissin Corporation in 1985.

Fig. 15.3 Quorn, a meat substitute

They developed a meat substitute which mimicked the taste and texture of meat. They developed a series of food products under the trade name of "Quorn" foods (Fig. 15.3). The patented technology was that of a mycoprotein derived from *Fusarium venenatum* fungus and was grown by fermentation process similar to the process of making beer or yogurt (https://en.wikipedia.org/wiki/Quorn (as accessed on 06-09-2017)). They launched their substituted meat product in the form of various prepackaged meals and cooking ingredients. Quorn is sold as an alternative for meat in a range of prepackaged meals and a cooking ingredient where the fungus is mixed with egg albumen and potato for vegetarian option as binder to get the desired texture (Finnigan, TJA (2011), Mycoprotein: origins, production, and properties, in *Handbook of Food Proteins* (eds. G. O. Philips and P. A. Williams), pp. 335–352, Woodhead Publishing Ltd. (accessed on 06-09-17)).

The technology involved in development of this brand was "production of edible protein containing substances" (US 4555485).

Pop Rocks

The novelty of the technology behind the candy was that it caused a fizzy reaction while dissolving in the mouth. The concept was patented by general foods research chemists Leon T. Kremzner and William A. Mitchell on December 12, 1961. Later Kraft Foods licensed the Pop Rocks brand to Zeta Espacial SA who continued manufacturing the product under Kraft's license and later became the sole manufacturer and owner of the brand (http://www.pop-rocks.com/ (as accessed on 06-09-2017)).

"Gasified confection and method of making the same" (US 3012893) the patented technology behind the famous American popping candy Pop Rocks) (Fig. 15.4).

Fig. 15.4 American popping candy

Fig. 15.5 Uncrustables

Sealed Crustless Sandwich

JM Smucker Company started making these sealed crustless sandwiches using the brand name "Uncrustables." The patent was challenged in court by a grocery and catering company in Gaylord, Michigan, called Albie's Foods, Inc. which was selling a crimped-edged no crust pocket sandwich called a "pasty" and was served a cease and desist notice by JM Smucker. The reexamination of the "sealed crustless sandwich" by USPTO showed a number of prior art existed for the same which were neglected previously. Eventually the patent was rejected along with any claims; however, JM Smucker continued to sell the unpatented sandwiches under the trademark of "Uncrustables" (https://en.wikipedia.org/wiki/Sealed_crustless_sandwich (as accessed on 06-09-2017)) (Fig. 15.5).

This case was considered as a controversial one because of the haste and carelessness shown by the patent office. The patent office without properly examining patent application and conducting complete prior art search granted the patent, although patents for similar products already existed (Adam B. Jaffe and Josh Lerner, Innovation and its Discontents: How our broken patent system is endangering innovation and progress, and what to do about it (ISBN 0-691-11725-X; Princeton, NJ: Princeton University Press, 2004), 25–26, 32–34 (as accessed on 06-09-2017)).

Copyright

According to WIPO "copyright is a legal term used to describe the rights that creators have over their literary and artistic works." Although the US Copyright Law extends protection to "original work of authorship fixed in any tangible medium of expression," it grants authors exclusive privilege to produce, create, or display such work.

Copyrights in food and beverages are not well outlined and has many overlapping gray areas. The copyright for recipes is debatable as they are merely a list of ingredients or a method of cooking is not enough to secure protection under copyrights. Expression of original recipe instructions in the form of a book or any other forms of compilation can always be a subject matter of copyright. However, copyright protection can cover certain ingredients and their quantities, the idea of the product, and the style, method, or technique of preparation etc. (http://www.wipo.int/copyright/en/ (as accessed on 31-03-2017)).

Fierce competition and entry of large number of international brands in a market due to globalization have resulted in an immense growth in advertising and marketing to capture a larger market share. This has resulted in organization utilizing unethical ways in order to gain an edge over their competitors, sometimes resulting in financial losses to those who have put in great efforts and skills to obtain goodwill for their brand or product. Here copyright laws play a pivotal role in safeguarding all the aspects of a creation like advertising, slogans, script, etc.

Advertisements and Copyright

Like others, the creative or artistic work advertisements should also meet certain criteria to be protected under copyright law. The artistic work should be:

1. Original – had not been copied previously.
2. It should not be in public domain or common knowledge.
3. The work should be in tangible expression and not merely an idea. Idea has no copyright.
4. The work must involve labor, skill, and capital.

As per section 13 of the Copyright Act, 1957, copyright protection is given to categories of work that are:

(a) Original literary, dramatic, musical, and artistic works
(b) Cinematographic films
(c) Sound recording

Advertisement usually is a culmination of literary, dramatic, artistic, and musical skills. But an advertisement can only be protected under copyright law only if fulfills the above four requirements (http://www.wipo.int/copyright/en/ (accessed on 31-03-2017)).

Below, few case studies are presented showing how copyrights of one party were infringed upon by another.

Pepsi vs. Coca-Cola (The Cola War)

Pepsi Co. and Coca-Cola both are giants in the cola segment and often are at war against each other on various aspects of intellectual property protection. A similar example is shown here, whereby Pepsi filed a suit against Coca-Cola for violating their trademark in a commercial.

In the commercial of "Thums Up," a product of Coca-Cola Company, a leading actor of Indian cinema is seen asking a kid as to which is his favorite drink. The kid replies Pepsi although muted but the lip movement indicating the same. Then the kid is asked to taste two bottles of cola where labels are covered, and the kid replies that the children will love the first brand because it is sweet, but he himself will prefer the second bottle as that drink is meant for grown-ups and is stronger. The actor then removes the label showing that the first bottle is named "Pappi" which is deceptively similar to Pepsi, and the second bottle is Thums Up. A further series of slogans followed like "wrong choice baby" and "Dil maange no more"; both were degrading the slogans of Pepsi like "Yeh hi hai right choice baby" and "Dil maange More" causing damage to Pepsi's reputation in the market which consisted of mainly young people mostly teenagers. The court ruled in favor of Pepsi stating that Coca-Cola has disparaged and depreciated the products of Pepsi by using deceptively similar globe logo and the word "Pappi" which is resembling to the trademark Pepsi (Semila Fernandes/Procedia – Social and Behavioral Sciences 133 (2014) 346 – 357 (accessed on 06-09-2017)).

Britannia v. Unibic Biscuits India

Britannia Good Day Biscuits have established a name for themselves and certain market goodwill as well. So when Unibic Biscuits launched a similar biscuit called "Great Day" along with a slogan saying "why have a Good Day when you can have a Great Day!" This campaign was a direct indication to Britannia Good Day suggesting that people should not consume "Good Day" Biscuits but prefer "Great

Day" Biscuits. The court ruled in favor of Britannia and granted an injunction to the defendant for depreciating "Good Day" Biscuits by exaggerating the facts and making an impression that no other facts hold true. Thus, conveying a wrong message to the public (Semila Fernandes/Procedia – Social and Behavioral Sciences 133 (2014) 346 – 357 (as accessed on 06-09-2017)).

Trademark

According to WIPO, "a trademark is a distinct sign or recognizable, design or expression which identifies products or services of a particular source from those of the others. Trademarks may consist of words, letters or numerals, drawings, symbols, three-dimensional features such as the shape and packaging of goods, non-visible signs such as sounds or fragrances, or color shades used as distinguishing features" (http://www.wipo.int/trademarks/en/ (as accessed on 31-03-2017)).

When it comes to food and beverages industry, trademarks play a substantial role in creating a brand and ensuring that the product is distinctive to the brand and thus creating a market value for that product.

Over the years we have seen certain trademarks establishing recognition and reputation for themselves and in turn have become extremely valuable to the companies since it is the single largest source of intangible value. Sometimes mere color or shape or logo or merely the shape of the product gives the brand its distinctiveness. Some trademarks which have distinguished themselves and established a strong consumer and market share are shown in Fig. 15.6.

Fig. 15.6 Turquoise color of Heinz, Coca-Cola logo, and shape of Toblerone chocolates as trademarks

With the ever-expanding and competitive food and beverages industry, the companies spend enormous resources in launching a new product and building a compelling brand that attracts customers, thus maintaining their steady consumer base. But sometimes smaller brands with careful planning try to harness the power of a successful brand by launching deceptively similar product or packaging. A brand and its goodwill are extremely valuable; for example, about half the entire value of the Coca-Cola Company is its brand, which was estimated in 2011 to be worth $74 billion. So it is worthwhile for companies to protect their brand via trademark registration. From time to time, certain trademark infringement proceedings which form basis of certain landmark judgments come to light. Some of them are discussed here.

Sugar-Free Case-Determining Secondary Distinctiveness of a Trademark

Sometimes the strength of a trademark over the years has increased so much that in consumer mind trademark is associated with a particular product or manufacturer or service, which are often used interchangeably.

Therefore, as in Cadila Healthcare vs. Gujarat Cooperative Milk Marketing Federation Ltd., we see that determining secondary distinctiveness is not easy. Cadila were selling and marketing their product Aspartame, an artificial sweetener as a low-calorie tabletop sweetener, which is as sweet as sugar containing only 2% of its calories, under the trademark "Sugar-Free." Gujarat Coop Society started marketing their ice cream under the trademark Sugar-Free D'lite. The plaintiffs filed a suit for infringement at the Delhi High Court before a single judge refused to grant interim injunction. The division bench of the Delhi High Court, before whom the appeal lays, inter alia, observed that the mark "sugar-free" may be distinctive of an artificial sweetener but cannot be distinctive from other goods. It might be specified here that the litigant had a 74% offer in the sugar substitute market in India, plainly building up a prevalent premium. The Hon'ble Division Bench held that the mark "sugar-free" is descriptive in nature and was not a coined word or an irregular combination of words; hence plaintiff cannot claim exclusive right over it.

However the Gujarat Cooperative were ordered by the court to reduce or make less prominent the font size of the term "sugar-free," which perceptibly stood bigger than the trademark of the product "Amul." Here we conclude that although the term "sugar-free" as a trademark has acquired secondary distinctiveness for Cadila in the market, Cadila cannot prevent the use of the term "sugar-free" in its descriptive sense (https://indiankanoon.org/doc/156583015/ (accessed on 06-09-17)).

Kellogg Inc. v. National Biscuit Co.

The process of making shredded wheat biscuit was developed by Henry Perky who later introduced the product in the market in 1963. He was granted utility patent on product as well as the machinery used to make shredded whole wheat biscuits.

Fig. 15.7 Shredded wheat (Nabisco) and shredded wheat (Kellogg's)

They were marketed under the name of "Shredded Whole Wheat" and later "Shredded Wheat."

In 1912, after the patent of Henry Perky expired, the Kellogg Company started making wheat biscuit. Although the Kellogg's biscuits were similar in form to shredded wheat but were made from a different process than that of Henry Perky's shredded wheat. The Shredded Wheat Company the successor to Perky's company objected to Kellogg's product, and thus Kellog stopped its manufacturing in 1919. But later resumed it in 1927 but were sued by National Biscuit Company (later called Nabisco) who had acquired Shredded Wheat Company. Nabisco objected in their lawsuit the term "shredded wheat," the similarity of the shape of their biscuits to that of Kellogg's and the use of picture of two pillow-shaped cereals submerged in milk on their cereal box (Fig. 15.7) (Graeme B. Dinwoodie, "The Story of KELLOGG CO. v. NATIONAL BISCUIT CO.: Breakfast with Brandeis," p. 1, retrieved on 15 may 2017 (as accessed on 06-09-2017)).

The US Supreme Court rejected Nabisco's arguments stating that since the patent of the technology and product has expired, Kellogg's have a right to copy. As to the picture on cereal box, the court stated that since the word Kellogg's was so prominently displayed on the cereal box, the consumer cannot be confused between the two brands. Thirdly the term "shredded wheat" was a generic term and therefore not trademarkable (https://en.wikipedia.org/wiki/Kellogg_Co._v._National_Biscuit_Co. (as accessed on 06-09-17)).

Although the court agreed that Kellogg was sharing in the goodwill created by Nabisco, that was not unfair once the article is not protected by patent or trademark.

Color as Trademark (Fig. 15.8)

Cadbury was given a royal warrant in 1854 by Queen Victoria, upon which it became the Queen's official cocoa and chocolate maker. As a tribute to Queen Victoria, Cadbury filed a trademark application for its legendry color purple also

Fig. 15.8 Color purple of Cadbury as trademark

Fig. 15.9 Design of Coca-Cola bottle

known as Pantone 2685C in 2004. Nestle the main market rival of Cadbury filed objection to the registration of color purple as trademark stating that the color purple was commonly used in trade and does not indicate Cadbury merely on the basis of color purple. The US Court rejected Cadbury's application thus rejecting Cadbury's attempt to monopolize a shade of purple for its milk chocolate products (http://www.azrights.com/media/news-and-media/blog/branding/2016/04/high-court-rules-cadburys-purple-trade-mark-is-not-a-series-mark/ (as accessed on 06-09-2017)).

This case indicates that trademarks should be clear and descriptive. This should particularly be taken into consideration when the trademark applied for is of unusual nature, like color or odors as trademark. Tiffany having successfully registered Pantone 1837 paves way for many such as applications of color as trademark. This indicates how trademark laws are constantly being stretched and challenged as innovative ideas of brand distinction emerge which are at a different course than the more conservative modes of protecting a brand.

Industrial Design

In legal analogy, an industrial design constitutes the ornamental or esthetic aspect of an article or product. It has got nothing to do with the functionality of an article.

An industrial design may consist of three-dimensional features, such as the shape of an article, or two-dimensional features, such as patterns, lines, or color.

One such example is the design of Coca-Cola bottle (Fig. 15.9).

In industrial design registration also called design patent, the owner has the right to prevent a third part from making, selling, or importing articles bearing or incorporating a design which is a copy of the registered design. Protection is provided to the new nonfunctional esthetic features of colors, ornamentation, configuration, pattern, or composition of lines or shape. A design can be two- or three-dimensional patterns used to produce a product, article, or handicraft through industrial process manually, mechanically, or chemically, applied separately or in combination. Indian Design Act, 2000 was established and implemented to comply with Articles 25 and 26 of Trade-Related Aspects of Intellectual Property Rights (TRIPS) agreement. The registration provides protection up to 10 years and is renewable for another 10 years. Industrial design is country specific like patents. WIPO administers certain treaties, together with regional and national laws which form the backbone of the legal structure of industrial designs. Some of the industrial design-related treaties are:

- Hague Agreement for the international registration of industrial designs allows the owner to file only one application and in one language and one fee to obtain protection in several countries.
- Global Design Database by utilizing multiple databases of Hague System and other participating nationals provides a large collection of registered design patents.
- Locarno Classification is an international system to classify goods for the purpose of industrial design registration.

Coke vs. Pepsi: "The Great Bottle Battle"

The "contour bottle" of Coca-Cola is one of the most recognizable shapes among people. The fluted line bottle was designed by Earl R. Dean after winning a competition in 1915 launched by Coca-Cola urging its bottle suppliers to come up with a design that would establish the singularity of the design with Coke, thus making it distinct from other brands in the market. In 2007 Pepsi launched its "Carolina Bottles" in Australia (Fig. 15.10). Coke filed a suit in court against Pepsi for trademark infringement, misleading and passing off. In 2014 the court rejected all the claims of Coca-Cola against Pepsi after carefully examining both the bottles and stating that the outline or silhouette was just one of the elements of the design and not the complete representation of "contour bottles." Other than that the Pepsi bottles differed in many other aspects like being more curvaceous, fluting patterns on top and bottom parts, and differences in base and neck designs (http://www.patentadesign.com/gallery/coca-cola-bottle-design-patent.html. Accessed on 06-09-17).

This is a case of shape trade where the shape of the bottle was synonym with the recognition of the product. In shape designs the specifications should be in detail along with the essential features of trademark (https://en.wikipedia.org/wiki/Coca-Cola#Contour_bottle_design (as accessed on 06-09-17)).

Fig. 15.10 Comparison of contour and Carolina bottles

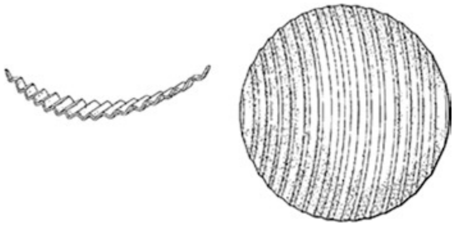


Fig. 15.11 The wavy lays potato chip

The Wavy Lays Potato Chip (Fig. 15.11)

Frito-Lays' innovative design patent (UD D495, 852 S) of the Wavy Lays, or Ruffles, seemed to be an exquisite market strategy with the company claiming that the ridges make the chip tastier. Tasty or not can be contentious, but it definitely provides the chip a singularity over other chips in terms of brand recognition. Ridged chip even without its packaging can be related to Frito-Lays. This simple innovation in design has established Frito-lays as leaders in chips manufacturer and eliminating competition by providing exiting looking chips (http://www.patentadesign.com/gallery/frito-lay-wavy-lays-potato-chip-design-patent.html as accessed on 06-09-17)).

The Kellogg's Eggo Waffle

The success of this classical American breakfast, "The Waffle," is due to its unique design and conception distinguishing it from any other ordinary pancakes (Fig. 15.12). The Kellogg's Eggo Waffle's unique design of cubicle-like compartments may just seem esthetic, but they also provide functionality to the waffle. The cubicle structure holds syrup making the waffle tastier than ordinary pancake. This, along with a strong television campaign of "Leggo my Eggo," has made this a market and consumer success (http://www.werkerlaw.com/tradesecrets.htm#CONCLUSION (as accessed on 06-09-2017)).

Fig. 15.12 The Kellogg's Eggo Waffle

Trade Secret

Any confidential commercial information which gives a competitive edge may be considered as a trade secret. Sometimes the product is so successful that the protection offered by patents for 20 years is not sufficient, and therefore the owner of the knowledge may choose to keep the information confidential as trade secret and may reap the profits of the trade indefinitely. In the food industry, the trade secrets may range from the ingredients of the recipe or method of preparation and even a sales and marketing strategy.

Despite the facts that trade secrets provide unlimited protection as long as the secret is protected, its major drawback is that it cannot stop third parties to work out a way to dissect the product and use it commercially.

Among the best kept trade secrets in the world, a large number do belong to the food and beverages industry. The formula of Coke (Merchandise 7X) is considered to be the best-kept trade secret and may be the longest. Other prominent and commercially successful trade secrets are KFC fried chicken, Dr Pepper, and Bush's Baked Beans. Selling your product as a trade secret provides a mysterious tinge to the product making this an excellent marketing strategy and advertising campaign.

Any organization or an entity irrespective of its size possesses within itself information and knowledge which is paramount for its growth and to maintain its position in the market. Rapid economic growth and involvement of various internal and external sources (outsourcing of services, etc.) makes it difficult to contain the spillage of important knowledge or information. IPR tools like patents, trademarks, copyright, and design may protect some of the aspects of an organization. But there are certain façades which cannot be covered by IPR tools, and here the trade secrets play pivotal role in protecting them. Particularly in the food and beverages industry, trade secrets are a paramount to the taste or appearance of a product.

Coca-Cola

As one of the best-selling beverage companies with an equity value of over 100 billion dollars, Coca-Cola has established itself as a well-known brand like no other. John Pemberton created the formula of Coca-Cola in the late nineteenth century and patented it in 1893 disclosing the formula of the recipe. But later when company changed the formula of their carbonated drink, they choose not to patent it but rather to keep it as a trade secret. There were various reasons behind it. Firstly, by the twentieth century, Coke had established itself as a strong brand with very few competitors. Patenting the formula would provide them protection for only 17 years under the Patents Act, 1836, whereby they would be forced under the act to disclose their recipe, and after the expiration of the patent, anyone was free to manufacture the beverage. They knew that their brand and product value were way more than 17 years. Their policy on trade secret was so stringent that they decided to leave Indian consumer market. In 1977 they were required to disclose their formula under Indian Foreign Exchange Regulation Act (FERA). It only came back when India decided to change its policy after an absence of 17 long years. From time to time, there were efforts to steal the recipe of Coke, but this only added to the value of the brand and may be considered by some as quick market strategy to maintain the mystique image of their product (http://bdjls.org/coca-colas-secret-formula-trade-secret-kept-century/ (accessed on 06-09-17)).

Faccenda Chicken Ltd. vs. Fowler [1987]

The responsibility of keeping the trade secret lies solely with the owner of the product or process. The organization must ensure through legal agreements between employees, and the organizations have to be well defined in order to safeguard the trade secret.

Faccenda employed Fowler as sales manager for their operation where fresh chicken was sold from refrigerated vans in designated areas. Each salesman had knowledge of their customer base, their addresses, and price paid by them. The first defendant Fowler subsequently resigned from the plaintiff's, i.e., Faccenda's, employment and formed his own company to carry on a similar business in the same area. Eight of the company's employees went to work with the former employee. The company alleged that the employees have breached their employment contract by disclosing sales information of Faccenda company customer base. On examination it was found that none of the legal agreements between the company and the employees suggested that they cannot utilize the knowledge they acquired during their employment after leaving the services of their employee. The court differentiated the knowledge acquired by the employee during its tenure into three categories.

1. Information which was in public domain and does not require legal agreements and can be imparted to anyone.
2. Classified information which he cannot divulge while he is in employment, but in the absence of any binding agreement, he may utilize the information once he leaves the organization.
3. A trade secret to which he is bound by legal agreements and cannot divulge it during and after the tenure of employment.

The court held that since the present case fell under second category and there was no mention of it in the contract, therefore there was no breach once the employee left the employment and use the information gained during the course of his employment for his own benefit. He accordingly dismissed the plaintiff's action and counterclaim; therefore it is paramount for the employer to safeguard their intangible assets by strong legal agreements at the same time allowing the employee to utilize their skills without fear of any legal proceedings against them (http://www.werker-law.com/tradesecrets.htm#CONCLUSION (as accessed on 06-09-2017)).

Geographical Indication

A geographical indication (GI) is a sign used on products that have a specific geographical origin and possess qualities or a reputation that are due to that origin. Since the qualities depend on the geographical place of production, there is a clear link between the product and its original place of production (http://www.wipo.int/geo_indications/en/ (as accessed on 31-03-2017)).

Table 15.1 represents some of the GIs registered in India associated with food and beverages industry (https://en.wikipedia.org/wiki/List_of_Geographical_Indications_in_India (as accessed on 31-03-2017)).

In World Trade Organization's agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS), protection provided by geographical indication (GI) has been one of the most debatable issues in the IPR basket. According to TRIPS a geographical indication indicates a product having its origin to a specific geographical area, whereby because of its geographical conditions the product has developed some unique characteristics that have distinguished it from similar products from a different geographical area. The product should also have a certain reputation.GI does not provide protection to an individual but is a community or regional right. The right provides exclusivity to its recipients to use the given name for their product. The Geographical Indications of Goods (Registration and Protection) Act 1999 protects the GIs in India. Although registration is not compulsory in India, registration provides a legal stronghold for defending and economizing the GI. Since a reputation is attached to a product, the value of the product also depends on it. It is advisable to register GI to prevent unethical business practices like passing off and misleading to consumers. Also there is revenue loss due to unfair business practices. GI not only provides protection to a product and helps to generate revenue but also helps the entire community involve in terms of employment and safeguarding certain lost arts and techniques by creating awareness in the market for these products.

Table 15.1 Some of the GIs registered in India associated with food and beverages industry

S. no	Geographical Indication	State
1	Nagpur orange	Maharashtra
2	Darjeeling tea	West Bengal
3	Naga Mircha	Nagaland
4	Fenni	Goa
5	Mango Malihabadi Dusseheri	Uttar Pradesh

Darjeeling Tea

The governing body over all teas produced in tea-growing areas is the Tea Board of India under the Tea Act 1953. Since Darjeeling tea enjoys such an international reputation, much of it is attributed to its sole control by Tea Board and its protection under the GI. Since reputation of Darjeeling Tea is of such epic proportions and at times represents the cultural heritage of India, it is in the best interest of the government to protect any misleading brand of tea of being passed off as Darjeeling tea. Therefore to maintain the authenticity of the region of origin of Darjeeling Tea, a logo was designed and registered for easy identification of the GI, popularly known as the "Darjeeling" logo (Fig. 15.13).

The various intellectual property rights attributed to Darjeeling tea and its logo are:

(i) The word Darjeeling and its logo are registered trademarks of Tea Board of India under the Trademarks Act 1999.
(ii) The words Darjeeling and its logo were the first to be registered under the Geographical Indications of Goods (Registration and Protection) Act 1999 in India.
(iii) The Darjeeling logo is protected under the Copyrights Act 1957 as an artistic work.

Apart from being registered in India as GI, it is also a certified trademark in the UK, the USA, Belgium, Canada, Italy, Switzerland, Egypt, Yugoslavia, Germany, Lebanon, Austria, Spain, France, Portugal, Japan, and Russia and a community collective mark in European Union (https://www.ipandbusiness.com/darjeeling-tea-indian-geographical-indication/ (as accessed on 06-09-2017)).

Tea Board, India, vs. ITC Limited

Despite having such strong intellectual property protection, this famous GI is also at times engaged in legal battles. One such dispute was between Tea Board of India and ITC limited over the use of word "Darjeeling."

Fig. 15.13 Darjeeling logo

ITC named one of its lounges "Darjeeling" at their hotel in Calcutta. Tea Board of India asserted exclusivity over the word "Darjeeling." ITC maintained that there was more meaning and symbolism attached to the word Darjeeling than just tea. The court ruled in favor of ITC stating that the Tea Board was only a certification body that determined whether a tea should be called a Darjeeling tea or not. Tea Board was not involved in hospitality business nor was ITC infringing on the certification granting rights of Tea Board. Therefore there was not a case of passing off (https://www.ipandbusiness.com/darjeeling-tea-indian-geographical-indication/ (as accessed on 06-09-2017)).

Scotch Whisky Association vs. Golden Bottling Ltd.

Scotch whisky is protected under the Scotch Whisky Association (SWA), a UK-based organization. A dispute evolved, whereby SWA filed a petition against Golden Bottling Limited which was manufacturing liquor by the name of "Red Scotch" Whisky.

The SWA claimed that the use of the term whisky was giving the market and consumers an impression that the whisky had originated in Scotland which was not the case. The court after analyzing the GI status "Scotch Whisky" said that since it was not registered as a GI in India at that time, the GI status could not be upheld. But under Section 20 (https://globaledge.msu.edu/industries/food-and-beverage/memo) of the Indian GI Act 1999, which prohibits any person from instituting any proceedings to prevent or to recover damages for the infringement of an unregistered GI, held the defendant guilty only of passing off and refraining them from the use of the term "Scot" or "Scotch" (https://indiankanoon.org/doc/1122965/ (accessed on 06-09-17)).

Acknowledgment The material discussed here in this chapter has been sourced from various websites for the purposes of education, dissemination of knowledge, and information.

The author sincerely renders gratitude to Ms Garima Pant and Ms Zeba Siddiqui, DST-India, TIFAC KIRAN-IPR interns for their significant inputs. Thanks are due to CSIR-CDRI, Lucknow.

References

Dinwoodie GB. The story of Kellogg Co. v. National Biscuit Co.: breakfast with Brandeis, p. 1. Retrieved on 15 may 2017. Accessed on 06-09-17

Finnigan TJA (2011) Mycoprotein: origins, production and properties. In: Philips GO, Williams PA (eds) Handbook of food proteins. Woodhead Publishing Ltd, Cambridge, pp 335–352. Accessed on 06-09-17

http://bdjls.org/coca-colas-secret-formula-trade-secret-kept-century. Accessed on 06-09-17

https://en.wikipedia.org/wiki/Coca-Cola#Contour_bottle_design. Accessed on 06-09-17

https://en.wikipedia.org/wiki/Drink. Accessed on 30-03-17

https://en.wikipedia.org/wiki/Kellogg_Co._v._National_Biscuit_Co. Accessed on 06-09-17

https://en.wikipedia.org/wiki/List_of_Geographical_Indications_in_India. Accessed on 31-03-17
https://en.wikipedia.org/wiki/Quorn. Accessed on 06-09-17
https://en.wikipedia.org/wiki/Sealed_crustless_sandwich. Accessed on 06-09-17
https://globaledge.msu.edu/industries/food-and-beverage/memo. Accessed on 30-03-17
https://indiankanoon.org/doc/1122965/. Accessed on 06-09-17
https://indiankanoon.org/doc/156583015/. Accessed on 06-09-17
http://www.azrights.com/media/news-and-media/blog/branding/2016/04/high-court-rules-cadburys-purple-trade-mark-is-not-a-series-mark/. Accessed on 06-09-17
http://www.fooddive.com/news/top-10-food-and-beverage-industry-trends-and-why-they-matter/404484/. Accessed on 30-03-17
https://www.foodsafety.gov/keep/types/. Accessed on 30-03-17
https://www.ipandbusiness.com/darjeeling-tea-indian-geographical-indication/. Accessed on 06-09-17
http://www.patentadesign.com/gallery/coca-cola-bottle-design-patent.html. Accessed on 06-09-17
http://www.patentadesign.com/gallery/frito-lay-wavy-lays-potato-chip-design-patent.html. Accessed on 06-09-17
http://www.pop-rocks.com/. Accessed on 06-09-17
http://www.werkerlaw.com/tradesecrets.htm#CONCLUSION. Accessed on 06-09-17
http://www.wipo.int/copyright/en/. Accessed on 31-03-17
http://www.wipo.int/geo_indications/en/. Accessed on 31-03-17
http://www.wipo.int/patents/en/. Accessed on 30-03-17
http://www.wipo.int/trademarks/en/. Accessed on 31-03-17
Jaffe AB, Lerner J (2004) Innovation and its discontents: how our broken patent system is endangering innovation and progress, and what to do about it. Princeton University Press, Princeton, pp 25–26. 32–34. ISBN 0-691-11725-X. Accessed on 06-09-17
Semila Fernandes/Procedia – Social and Behavioral Sciences 133 (2014) 346–357. Accessed on 06-09-17

Chapter 16
Application of Computational Intelligence Techniques for Forecasting Problematic Wine Fermentations Using Data from Classical Chemical Measurements

Gonzalo Hernández, Roberto León, and Alejandra Urtubia

Abstract The early forecasting of normal and problematic wine fermentations is one of the main problems of winemaking processes, due to its significant impacts in wine quality and utility. In Chile this is a critical problem because it is one of the top ten wine-producing countries. In this chapter, we review the computational intelligence methods that have been applied to solve this problem. Both methods studied, support vector machines and artificial neural networks, show excellent results with respect to the overall prediction error for different training/testing/validation percentages, different time cutoffs, and several parameter configurations. These results are of great importance for wine production because they are based only on measurement of classical chemical variables and they confirm that computational intelligence methods are a useful tool to the winemakers in order to correct in time a potential problem in the fermentation process.

Keywords Support vector machines · Neural networks · Wine fermentations · Classical chemical variables

G. Hernández (✉)
Universidad de Santiago de Chile, Departamento de Ingeniería Industrial, Santiago, Chile
e-mail: gonzalo.hernandez.o@usach.cl

R. León
Universidad Andrés Bello, Facultad de Ingeniería, Viña del Mar, Chile

A. Urtubia
Universidad Técnica Federico Santa María, Departamento de Ingeniería Química y Ambiental, Valparaíso, Chile

Centro Regional de Estudios en Alimentación Saludable, Valparaíso, Chile

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7_16

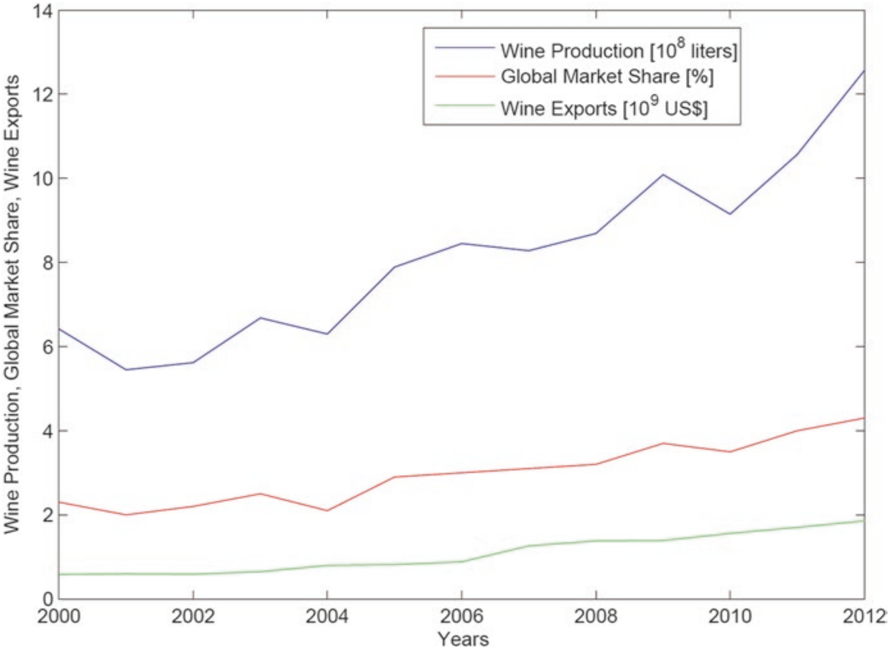


Fig. 16.1 Evolution of the production, exportation, and market share of Chilean wines from 2000 to 2012

Introduction

Between 2011 and 2016, the Chilean winemaking industry has produced approximately 70,000 hl of wine; see Fig. 16.1, which has positioned Chile as one of the top ten wine-producing countries and among the top five major wine-exporting countries (Executive Report Chilean Wine Production 2011). This success is mostly explained because of the excellent quality of the Chilean wine in relation with its price.

During the fermentation process, the wine quality can present problems that produce problematic fermentations: stuck or sluggish. A normal fermentation (called a dry fermentation) contains less than 0.2–0.4% residual sugar (Bisson and Butzke 2000). Stuck or incomplete alcoholic fermentations are defined as those leaving a higher than desired residual sugar content in the wine once the yeast has ceased to act, while a sluggish fermentation occurs when the yeast is struggling to ferment (late-onset), and it can potentially stop fermenting altogether and become stuck (Blateyron and Sablayrolles 2001; www.techniquesinhomewinemaking.com). In the case of Chile, approximately 1–3% of wine fermentation processes are problematic, but this range is imprecise and variable.

The current literature establishes that the main causes of problematic fermentations are the following: high initial sugar content, extreme temperature or pH, lack of nutrients such as nitrogen or oxygen, high ethanol content, competition from other microorganisms, short- and medium-chain fatty acids, and incorrect enological practices (Bisson and Butzke 2000; Blateyron and Sablayrolles 2001; Pszczólkowski et al. 2001; Beltran et al. 2008; Varela et al. 2004; D'Amatto et al. 2006) (Fig. 16.2).

In addition, problematic wine fermentations can produce significant economic impacts. For example, considering only losses in raw materials, the cost is approximately USD 43,200 per fermentation in a 32 tons fermenter.

Some of the fermentations can be corrected by enological procedures, and the fermentations can finish normally, with a lower quality because of the increased time of fermentation and the corrective actions that were taken. Therefore, due to the economical and quality impacts of abnormal wine fermentations, the early availability of low error problematic fermentation forecasts would enable winemakers to take timely corrective actions and significantly reduce winery and economical losses.

Usually, in the wine industry, alcoholic fermentation is monitored with standard measurements (density, reduced sugars, total and volatile acidity, pH, temperature, etc.) made daily. However, when its normal behavior is affected for some stress condition, and the fermentation could be stuck or sluggish, the action of the enologist is to try to correct the problem in the moment, with the previous information. Many times, it can be solved with the employment of traditional practices such as inoculations, nutrient additions, and others, but the question is why not tried to avoid them?

Computational Intelligence Techniques Applied to Wine Fermentation Problems

Computational intelligence refers to methods that come from artificial intelligence and that have been constructed using an approach based on learning and adaptation in order to solve complex pattern recognition problems. The methods most used are:

- Artificial neural networks (ANN)
- Support vector machines (SVM)
- Genetic algorithms (GA)

In general, the first two ones are applied in tasks that appear in pattern recognition, such as classification, identification, clustering, and regression, among others (Engelbrecht 2007; Kruse 2013; Bishop 1996, 2006; Theodoridis and Koutroumbas 2008; Abe 2010).

A multilayer feedforward artificial neural network (ANN) is a well-known mathematical model that can be characterized by several parameters, such as number of hidden layers; number of neurons of the input, output, and hidden layers, transition functions, and error function (Engelbrecht 2007; Bishop 1996; Theodoridis and

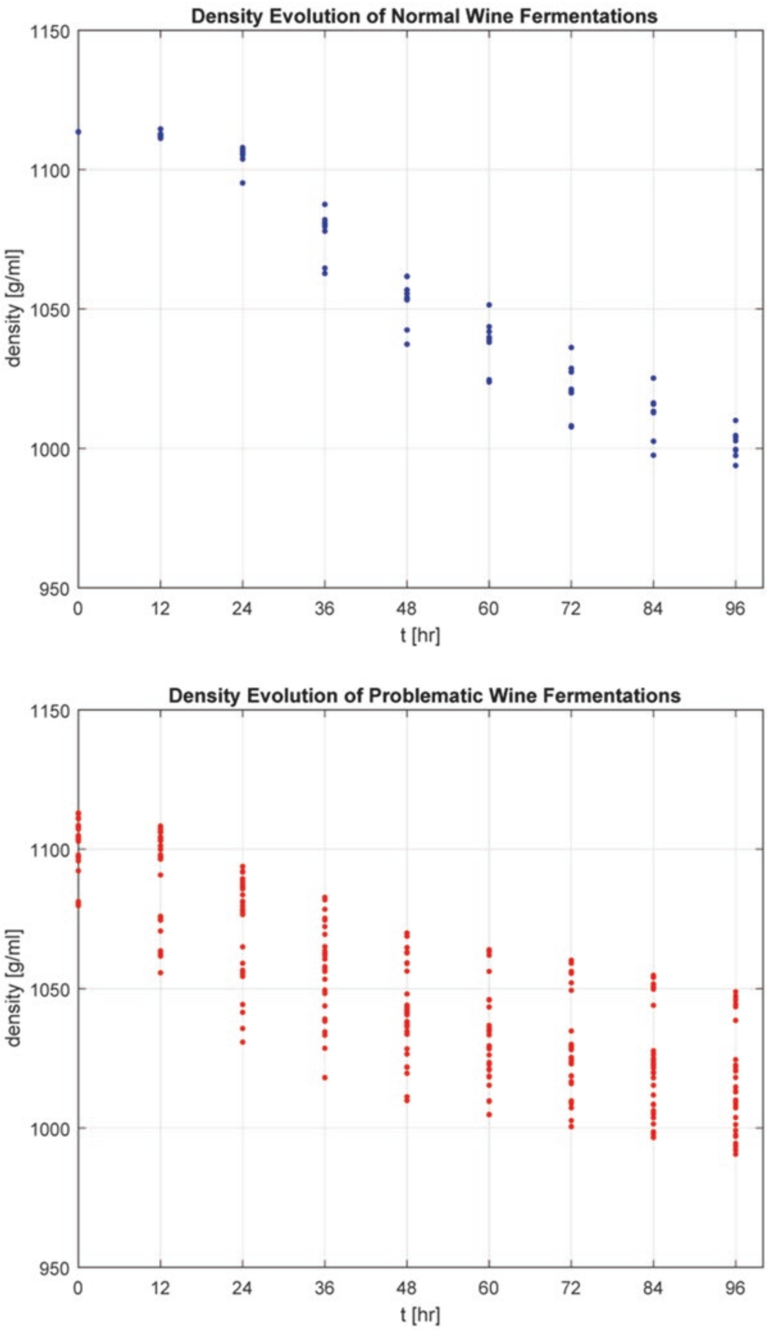


Fig. 16.2 Evolution of density in normal (8) and problematic (30) wine fermentations

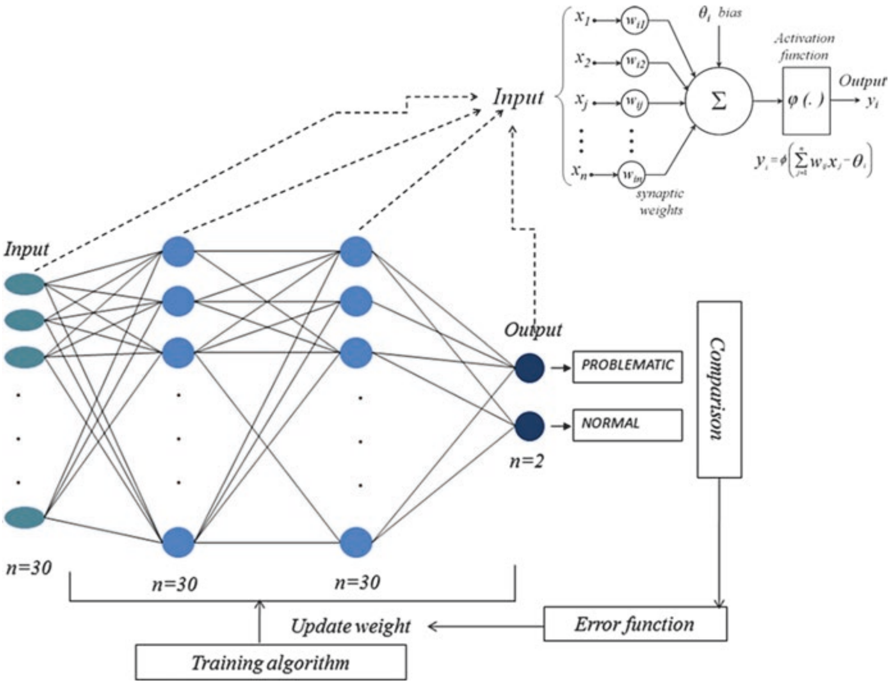


Fig. 16.3 Multilayer feedforward ANN with two hidden layers, like the one applied in (Urtubia et al. 2011)

Koutroumbas 2008; Ripley 2008). By the application of the learning rule, the ANN have shown in numerous cases coming from several areas the capacity to detect complex relationships between inputs and outputs; however this ability strongly depends on the correct specification of the ANN and the availability of a good number of examples (Bishop 1996, 2006) (Fig. 16.3).

In wine production, the applications of ANN have been concentrated in identification and classification task (Beltran et al. 2006; Marini et al. 2008; Penza and Cassano 2004; Perez-Magariño 2004; Kruzlicova et al. 2009; Hosu et al. 2014; Fernandes et al. 2015). Penza and Cassano (2004) chemometrically classified different classes of Italian wines using a multisensor array and different ANN methods. The best results obtained were a recognition and prediction rates of 100% and 78%, respectively. Both statistical and ANN methods were used to classify 70 Spanish rose wines with respect to their denominations of origin (Perez-Magariño 2004). By studying 19 variables, the SDLA statistical method chose 10 variables and obtained percentages of correct classification and prediction of 98.8% and 97.3%, respectively, while the ANN method chose 7 variables and obtained a correct classification for training and prediction of 100%. In Kruzlicova et al. (2009) a multilayer perceptron technique was applied in order to classify 36 Slovak wine samples of 3 varieties, produced by 4 producers in 3 different years with respect to the variables: variety, producer/location, and the year of production. In this case, considering different samples, the minimum prediction rate was 93.3%. In (Hosu et al. 2014), single-layer and probabilistic ANNs were developed for predicting valuable properties of wine antioxidant activity and for identifying the wine variety of grapes, harvest year, and originating winery. In Fernandes et al. (2015), results were presented by measuring pH, sugars, and anthocyanin content of Port wine grape berries by applying hyperspectral imaging and neural networks techniques.

Support vector machines (SVM) are also one of the most used methods to solve pattern recognition problems, specifically classification, identification, and regression (Abe 2010; Scholkopf and Smola 2002; Sánchez 2003). The mathematical formulation of SVM corresponds to an optimization problem whose solution computes the border that divides a data set in different classes. In the case of binary classification, the data set consists of $(n + 1)$ dimensional points $(x_1,y_1),\ldots,(x_m, y_m)$, where $x_i \in R^n$ and $y_i \in \{-1,1\}$. The SVM method solves the following optimization problem (Abe 2010; Scholkopf and Smola 2002; Sánchez 2003):

$$\begin{aligned} &\min_{w,b,\varepsilon}\left(\frac{1}{2}w^T w + c\sum_{m}^{i=1}\varepsilon_i\right) \\ &\text{s.t.} \\ &y_i\left(w^T\phi\left(x_i\right)+b\right) \geq 1-\varepsilon_i \quad \forall i=1,\ldots,m \\ &\varepsilon_i \geq 0 \quad \forall i=1,\ldots,m \end{aligned} \tag{16.1}$$

where the function $\varnothing(u)$ defines the kernel $K(u,v) = \varnothing^t(u)\varnothing(v)$. The most used kernels are linear, second- and third-degree polynomial, radial basis function, and sigmoid.

$$\begin{aligned} &\text{Linear:} && K\left(u,v\right) = u^T v \\ &\text{Polynomial with degree } d: && K\left(u,v\right) = \left(\alpha u^T v + \beta\right)^d, \quad \alpha > 0 \\ &\text{Radial Basis Function:} && K\left(u,v\right) = \exp\left(-\alpha u - v^2\right), \quad \alpha > 0 \\ &\text{Sigmoid:} && K\left(u,\text{v}\right) = \tanh\left(\alpha u^T v + \beta\right) \end{aligned} \tag{16.2}$$

The objective function in Eq. (16.1) minimizes the distance between borders that divide the different classes and the classification error. The constant $c > 0$ can be interpreted as a penalization parameter: a small value of c allows increasing the separation between borders, while a big value of c allows reducing the classification error. For the considered training and testing sets, the values of the parameters b,c are usually determined by exhaustive search over a grid of typical values of the parameters (b,c). However, some authors have studied different heuristics to improve the computation speed of the parameters (b,c) (Gaspar et al. 2012; Demyanov et al. 2012; Liao et al. 2015). The SVM method has been applied to different problems related to wine fermentation, such as determination of the rice wine composition by means of Vis-NIR spectroscopy (Yu et al. 2009), denomination of origin classification of Spanish white wines (Jurado et al. 2012), classification of grape varieties and evaluation of discriminatory capacities of family compound by the application of different machine learning methods (including SVM) (Gómez-Meire et al. 2014), and SVM calibration of total reflectance mid-infrared spectroscopy to monitor the evolution of Chinese rice wine fermentation (Wu et al. 2015).

In addition, different authors have proposed to use hybrid intelligent methods to solve pattern recognition problems (Liao et al. 2015; Nagata and Chu 2003; Rocha et al. 2007; Capparuccia et al. 2007; Yang et al. 2012). In this case, one method is used for the parameter optimization or architecture determination of another method, as well as for the training stage. For instance, in evolutionary neural networks, two hybrid methods based on ANN and evolutionary computation were applied for the construction and optimization of network architecture and connectivity matrices (Rocha et al. 2007). Both methods applied to real-world classification and regression problems show competitive performance with respect to other methods.

Application of Artificial Neural Networks and Support Vector Machines to Detect Problematic Wine Fermentation Using Data from Classical Chemical Measurements

The authors of this chapter have designed, implemented, and studied different methods coming from multivariate statistics and computational intelligence to early detect problematic wine fermentations (Urtubia et al. 2010a, b, 2011, 2012; Urtubia and Roger 2011; Hernández et al. 2016, 2017). In Urtubia et al. (2010a, 2012) and Urtubia and Roger (2011), the following statistical methods were applied: principal component analysis, multiway principal component analysis, clustering k-means, linear discriminant analysis, and multiway partial least squares. By laboratory-scale fermentations was built and studied a database that contains approximately 22,000 data which come from 22 normal, sluggish, and stuck fermentations of Cabernet Sauvignon wine. To obtain these data were measured, by mid-infrared spectroscopy and standard methods, 30–35 samples of each of the relevant chemical variables for each fermentation: sugars, alcohols, organic acids, nitrogen compounds, and density. The main result of Urtubia et al. (2010a, 2012) and Urtubia and Roger (2011) establishes that considering the measurements of the first 96 h of sugars, alcohols, and density, the statistical methods can predict, with low error, normal and problematic fermentations.

This result was improved in Urtubia et al. (2010b, 2011, 2012) by applying a multilayer perceptron ANN to timely detect the behavior of wine fermentations. It was used the same database of Urtubia et al. (2010a) and Urtubia and Roger (2011), and the ANN was defined with one or two hidden layers. The training algorithm was back-propagation with gradient descent, and the transition function was the sigmoid function. The training and testing sets were constructed randomly with 70% and 30% of the fermentations, respectively. Considering the main chemical variables, as in Urtubia et al. (2010a) and Urtubia and Roger (2011), it was computed the ANN prediction rates using data of the first 72, 96, and 256 h. By several computational experiments, it was demonstrated that ANNs can be applied to detect problematic wine fermentations at 72 h with excellent accuracy. However, the determination of the architecture of the ANN that produces the best prediction rates was a time-consuming and difficult process.

A similar methodology of (Urtubia et al. 2010b, 2011, 2012) was applied in (Hernández et al. 2016) but with a different prediction method. In this work the SVM method was studied in order to detect problematic wine fermentations with three different kernels: linear, third-degree polynomial, and radial basis function. For the training and testing phase, it was used the same database of Urtubia et al. (2010a, b, 2011, 2012) and Urtubia and Roger (2011). The sequential and parallel programs that implement the SVM methods compute the best prediction rates using the data of the first 48, 72, and 96 h of the three main chemical variables: total sugar, alcoholic degree, and density. For the definition of the training and testing sets was considered a random sample of the data set for different training and testing configuration: 60–40%, 70–30%, and 80–20%, determined with 95% confidence level and 3% estimated error.

In Table 16.1 are shown the best and worst prediction rates considering each training/testing percentages and each time cutoff obtained considering only the measurements of the main chemical variables. In Table 16.2, are shown the best and worst prediction rates obtained by each SVM method and its main statistics computed considering all training/testing percentages and time cutoffs.

The best and second best prediction rates were obtained for the 80[%]–20[%] and 70[%]–30[%] training and testing percentages, both results for polynomial kernels and 48 [h] time cutoff, 0.88 and 0.86, respectively. Considering all training and testing percentages and time cutoffs, the worst prediction rates are obtained in all cases by linear kernels and the best linear kernel result (0.72 at 70[%]–30[%] training/testing percentage and 24 [h] time cutoff; see Table 16.2) is significantly smaller than the best prediction rate. From these results we can affirm that the relation among the fermentation classes and chemical variables is not linear. Moreover, this relation can be represented more accurately by a third-degree polynomial or a radial basis kernel. If the performance of the SVM methods is observed on all training/testing percentages and time cutoffs, and not only in the best obtained results, it can be observed that the radial basis function kernel has a slightly better performance if the average, minimum, maximum, first, second, and third quartiles are considered. The main result of Hernández et al. (2016) establishes that the SVM method with third-degree polynomial and radial basis kernels predict correctly 88% and 85%, respectively. These results were achieved for an 80–20% training/testing percentage configuration and a time cutoff of 48 h. Therefore, this work improves the previous results obtained in Urtubia et al. (2010a, b, 2011, 2012) and Urtubia and Roger (2011).

Table 16.1 Best and worst prediction rates for different training/testing configurations and time cutoffs

Training/testing percentages	Best prediction rate (BPR)	SVM method of BPR	Time cutoff of BPR
80 [%]–20 [%]	0.88	POL	48 [h]
70 [%]–30 [%]	0.86	POL	48 [h]
60 [%]–40 [%]	0.83	RBF	48 [h]
Time cutoff	**Best prediction rate (BPR)**	**SVM method of BPR**	**Training/testing percentages of BPR**
24 [h]	0.82	RBF	80 [%]–20[%]
48 [h]	0.88	POL	80 [%]–20[%]
72 [h]	0.82	POL	80 [%]–20[%]
96 [h]	0.81	RBF	80[%]–20[%]
Training/testing percentages	**Worst prediction rate (WPR)**	**SVM method of WPR**	**Time cutoff of WPR**
80 [%]–20 [%]	0.61	LIN	72 [h]
70 [%]–30 [%]	0.58	LIN	72 [h]
60 [%]–40 [%]	0.57	LIN	72 [h]
Time cutoff	**Worst prediction rate (WPR)**	**SVM method of WPR**	**Training/testing percentages of WPR**
24 [h]	0.69	LIN	80[%]–20[%]
48 [h]	0.68	LIN	60[%]–40[%]
			80[%]–20 [%]
72 [h]	0.57	LIN	60[%]–40[%]
96 [h]	0.64	LIN	60[%]–40[%]
			70[%]–30[%]

In Hernández et al. (2017), it was studied the performance of artificial neural network as a method to timely predict problematic wine fermentations using the measurements (see Table 16.3) of the classical chemical variables (density, Brix, fructose, glucose, ethanol, and glycerol) obtained from laboratory-scale fermentations of cabernet sauvignon; eight of these were normal fermentations and the rest problematic (stuck and sluggish) with a total amount of 1476 measurements distributed in the following way:

- Density and Brix: 342 data for each chemical variable corresponding to 38 fermentations and 9 measurements every 12 h for each fermentation corresponding to the first 4 days of the process
- Fructose, glucose, ethanol, and glycerol: 198 data for each chemical variable corresponding to 22 fermentations and 9 measurements every 12 h for each fermentation corresponding to the first 4 days of the process

A program was developed in Matab© using the neural network pattern recognition toolbox for the computation of the overall prediction rate error for the classical chemical variables, considering only the measurements of a specific variable at one time. The program determines this error considering a network architecture with

Table 16.2 Best and worst prediction rates obtained by the SVM methods and its main statistics computed considering all training/testing percentages and time cutoffs

SVM kernel	**Best result**	**Training/test configuration**	**Cutoff**
Linear	0.72	70[%]–30 [%]	24 [h]
Polynomial	0.88	80[%]–20 [%]	48 [h]
Radial basis function	0.85	80[%]–20 [%]	48 [h]
SVM kernel	**Worst result**	**Training/test configuration**	**Cutoff**
Linear	0.57	60[%]–40 [%]	72 [h]
Polynomial	0.65	60[%]–40 [%]	72 [h]
Radial basis function	0.66	60[%]–40 [%]	72 [h]
Main descriptive statistics	**Linear kernel**	**Polynomial kernel**	**Radial basis function kernel**
Mean	0.66	0.75	0.78
Standard deviation	0.05	0.08	0.07
Maximum	0.72	0.88	0.85
Minimum	0.57	0.65	0.66
First quartile	0.61	0.67	0.72
Second quartile	0.68	0.73	0.81
Third quartile	0.69	0.82	0.82
80% percentile	0.71	0.82	0.83
90% percentile	0.71	0.86	0.83
95% percentile	0.72	0.88	0.85

Table 16.3 Sample of the data of normal and problematic fermentations

	Density	**[g/L]**			**Brix**	**[%m/v]**		
Ferm	$t = 0$ h	$t = 12$ h	$t = 24$ h	$t = 36$ h	$t = 0$ h	$t = 12$ h	$t = 24$ h	$t = 36$ h
F1	1114	1115	1106	1088	26.6	26.0	25.3	22.5
F2	1114	1113	1104	1080	26.6	26.1	25.3	21.5
F9	1113	1106	1077	1057	25.6	24.9	20.2	17.4
F10	1114	1107	1078	1053	25.5	24.7	20.1	16.4
	Ethanol	**[%v/v]**			**Glycerol**	**[ppm]**		
Ferm	$t = 0$ h	$t = 12$ h	$t = 24$ h	$t = 36$ h	$t = 0$ h	$t = 12$ h	$t = 24$ h	$t = 36$ h
F1	0.005	0.007	0.076	0.208	20.1	12.8	156.4	338.9
F2	0.005	0.041	0.706	4.394	20.1	359.5	1184.1	4967.2
F9	0.010	0.010	3.898	7.199	11.5	11.5	7094.4	9619.2
F10	0.086	0.750	4.029	6.976	170.8	1755.6	6703.4	10,617.8

only one hidden layer, the mean squared normalized error as performance function, and the update of the weights and biases by the Levenberg – Marquardt training function optimization. The overall prediction rate error is determined for five training/testing/validation configurations, 40%, 30%, 30%; 50%, 25%, 25%; 60%, 20%, 20%; 70%, 15%, 15%; and 80%, 10%, and 10%, with each set of examples chosen at random in a balanced way among all fermentations and for each training/testing/

Table 16.4 Overall prediction rate error (OPRE) statistics for the chemical variables considering the first 2 days of data

	First 2-day cutoff					
Chemical Vars./OPRE statistics	Density	Brix	Fructose	Glucose	Ethanol	Glycerol
Average	0.0226	0.1099	0.2539	0.2744	0.1579	0.0879
Std. deviation	0.0670	0.0958	0.1809	0.1540	0.1776	0.1158
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.4211	0.4474	0.7727	0.7273	0.6818	0.5455
Mode	0.0000	0.0263	0.1364	0.2273	0.0000	0.0000
Median	0.0000	0.0790	0.2273	0.2273	0.0909	0.0455
60% percentile	0.0000	0.1053	0.2273	0.2727	0.1818	0.0455
70% percentile	0.0000	0.1579	0.3182	0.3182	0.2273	0.0909
80% percentile	0.0263	0.2105	0.3636	0.3636	0.2727	0.1364
90% percentile	0.0263	0.2105	0.5727	0.5000	0.4363	0.2273

Table 16.5 Best overall prediction rate error (OPRE) of density, Brix, and glycerol for the 3- and 4-day cutoffs for each training/testing/validation configuration considering all the hidden layer sizes

	First 3-day cutoff			First 4-day cutoff		
Chemical variables Best OPRE results	Density	Brix	Glycerol	Density	Brix	Glycerol
Training/test./valid Configuration	80/10/10	70/15/15	80/10/10	70/15/15	70/15/15	70/15/15
Average	0.0166	0.0595	0.0825	0.0166	0.0702	0.0791
Standard deviation	0.0561	0.0643	0.1223	0.0561	0.0723	0.0948
Minimum	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Maximum	0.2105	0.2105	0.4091	0.2105	0.2105	0.3636
Mode	0.0000	0.0526	0.0000	0.0000	0.0263	0.0455
Median	0.0000	0.0526	0.0455	0.0000	0.0526	0.0455

validation configuration for several hidden layer sizes: from 4 to 30 neurons. In addition, there considered three different cutoffs for the measurements of the chemical variables: 2, 3, and 4 days./For instance, if the chemical variable is density, the data 2, 3, and 4 days consist of each fermentation in 5, 7, and 9 measurements, respectively.

Table 16.4 presents the main statistics of the overall prediction rate error obtained using the data of the first 2 days. Table 16.5 presents the main statistics of the overall prediction rate error obtained for the chemical variables with best results using the data of density, Brix, and glycerol, using the first 3 and 4 days of data. The statistics are computed considering all the training/testing/validation configurations and hidden layer sizes.

The results of (Hernández et al. 2017) establish that, from the point of view of the ANN method and the overall prediction rate error, the best classical chemical variables to timely forecast wine fermentations are density, Brix, and glycerol, in that order. This result is especially important mainly for the following reasons:

- These chemical variables are the easiest to measure in laboratory or production conditions.
- The total amount available of data of the wine fermentations (1476 measurements) is very small compared to other applications of ANN.
- It is possible to apply the ANN method to predict with great accuracy normal and problematic wine fermentations using only the data of the first 2 days of one of the following chemical variables: density, Brix, and glycerol. It is important to point out that these data consist only in 190, 190, and 110 measurements, respectively.
- The overall prediction rate error improves if data of the first 3 or 4 days are considered instead of the data of the first 2 days, in the sense that its maximum value decreases as the time cutoff increases.
- The 50–90% percentiles allow us to affirm that the results are robust in the sense that several values of the ANN parameters (training/testing/validation configurations and hidden layer sizes) obtain very small overall prediction rate error.

Conclusions, Gaps, and Future Research Needs

The great impact of problematic wine fermentations in profitability and quality of the process can be significantly reduced by increasing the knowledge and understanding of how the data measured from the time evolution of the chemical variables can be used to generate a timely and accurate forecast of normal and problematic wine fermentations. Especially important is to use the data of classical chemical variables because they are the simplest and cheapest to measure.

In this chapter, we have presented the main results of the application of computational intelligence methods to solve this problem, using the measurements of laboratory-scale wine fermentations of one of the classical chemical variables at one time: density, Brix, fructose, glucose, ethanol, and glycerol. The methods studied, support vector machines and artificial neural networks, show excellent results with respect to the overall prediction error for different configurations of its main parameters, improving the previous results obtained. Especially outstanding are the results obtained for the artificial neural networks methods considering the density chemical variable: an average of 0.0226 overall prediction rate error considering different training/testing/validation configurations, hidden layer sizes, and time cutoffs. This result is of main importance because of the small quantity of data used.

However, there are still important open problems in wine fermentation processes that have not been tackled properly, namely:

- Determination of the chemical variables that produce the most accurate and timely forecast of normal and problematic wine fermentations
- Construction of a mathematical or computational model for representing the interactions between the main chemical variables that intervene in the correct evolution of wine fermentation processes

- Determination of how the initial conditions of a wine fermentation affect the development of the process
- Construction of mathematical or computational model for representing the influence of the initial conditions of a wine fermentation in the outcome of the process

Therefore, we propose to apply computational intelligence methods to the former scientific problems due to the excellent results obtained for the problem studied in this chapter. The main pitfall of this study will be the usual one, by means of laboratory-scale wine fermentations to obtain sufficient quantity of good-quality data of the main chemical variables of this kind of process: density, sugars, alcohols, amino acids, organic acids, and saturated and unsaturated fatty acids.

Acknowledgement Research supported by grants: FONDECYT 1120679 and Conicyt PIA/Basal FB0821.

References

Abe S (2010) Support vector machines for pattern classification. 2nd edn. Springer Academic Press, Elsevier, Oxford, United Kingdom

Beltran N et al (2006) Feature extraction and classification of Chilean wines. J Food Eng 75:1–10

Beltran G, Novo M, Guillamón J, Mas A, Rozés N (2008) Effect of fermentation temperature and culture media on the yeast lipid composition and wine volatile compounds. Int J Food Microbiol 121:169–177

Bishop CM (1996) Neural networks for pattern recognition. Oxford University Press, Oxford

Bishop CM (2006) Pattern recognition and machine learning. Springer, New York

Bisson L, Butzke C (2000) Diagnosis and rectification of stuck and sluggish fermentations. Am J Enol Vitic 51(2):168–177

Blateyron L, Sablayrolles JM (2001) Stuck and slow fermentations in enology: statical study of causes and effectiveness of combined additions of oxygen and diammonium phosphate. J Biosci Bioeng 91(2):184–189

Capparuccia R, De Leone R, Marchitto E (2007) Integrating support vector machines and neural networks. Neural Netw 20:590–597

D'Amatto D, Corbo M, Del Nobile M, Sinigaglia M (2006) Effects of temperature, ammonium and glucose concentrations on yeast growth in a model wine system. Int J Food Sci Technol 41:1152–1157

Demyanov S, Bailey J, Ramamohanarao K, Leckie C (2012) AIC and BIC based approaches for SVM parameter value estimation with RBK kernels. JMLR W&CP 25:97–112

Engelbrecht AP (2007) Computational intelligence: an introduction. 2nd ed. John Wiley & Sons, West Sussex, England

Executive Report Chilean Wine Production (2011–2016) Servicio Agrícola y Ganadero de Chile

Fernandes AM et al (2015) Brix, pH and anthocyanin content determination in whole port wine grape berries by hyperspectral imaging and neural networks. Comput Electron Agric 115:88–96

Gaspar P, Carbonell J, Oliveira JL (2012) On the parameters optimization of support vector machines for binary classification. J Integr Bioinform 9(3):201

Gómez-Meire S et al (2014) Assuring the authenticity of northwest Spain white wine varieties using machine learning techniques. Food Res Int 60:230–240

Hernández G, Leon R, Urtubia A (2016) Detection of abnormal wine fermentation processes by support vector machines. Cluster Computing. 19(3):1219

Hernández G, Leon R, Urtubia A (2017) Application of neural networks for the early prediction of problematic wine fermentations using data from the classical chemical measurements, submitted to Journal of Biotechnology, (in press)

Hosu A, MirceaCristea VM, Cimpoiu C (2014) Analysis of total phenolic, flavonoids, anthocyanins and tannins content in Romanian red wines: prediction of antioxidant activities and classification of wines using artificial neural networks. Food Chem 150:113–118

Jurado M et al (2012) Classification of Spanish DO white wines according to their elemental profile by means of support vector machines. Food Chem 135(3):898–903

Kruse R, et al (2013) Computational intelligence: a methodological introduction. Springer-Verlag, London, England

Kruzlicova D et al (2009) Classification of Slovak white wines using artificial neural networks and discriminant techniques. Food Chem 112:1046–1052

Liao O et al (2015) Parameter optimization for support vector machine based on nested genetic algorithms. J Autom Control Eng 3(6):507–511

Marini F, Bucci R, Magri A (2008) Artificial neural networks in chemometrics: history, examples and perspectives. Microchem J 88:178–185

Nagata Y, Chu K (2003) Optimization of a fermentation medium using neural networks and genetic algorithms. Biotechnol Lett 25:1837–1842

Penza M, Cassano G (2004) Chemometric characterization of Italian wines by thin-film multisensors array and artificial neural networks. Food Chem 86:283–296

Perez-Magariño S et al (2004) Comparative study of artificial neural network and multivariate methods to classify Spanish DO rose wines. Talanta 62:983–990

Pszczólkowski P, Carriles P, Cumsille M, Maklouf M (2001) Reflexiones sobre la madurez de cosecha y las condiciones de vinificación, con relación a la Problemática de fermentaciones alcohólicas lentas y/o paralizante en Chile, Facultad de Agronomía, Pontificia Universidad Católica de Chile

Ripley BD (2008) Pattern recognition and neural networks. Cambridge University Press, Cambridge, USA

Rocha M, Cortez P, Neves J (2007) Evolution of neural networks for classification and regression. Neurocomputing 70:2809–2816

Sánchez D (2003) Advanced support vector machines and kernel methods. Neurocomputing

Scholkopf B, Smola AJ (2002) Learning with kernels: support vector machines, regularization, optimization, and beyond. The MIT Press, Cambridge, USA

Theodoridis S, Koutroumbas K (2008) Pattern recognition, fourth edition. Academic Press, Elsevier, San Diego, USA

Urtubia A, Roger JM (2011) Predictive power of LDA to discriminate abnormal wine fermentations. J Chemom 25(7):382–388

Urtubia A, Emparan M, Almonacid S, Pinto M, Valdenegro M (2010a) Application of MPCA and MPLS on industrial batch bioprocesses. J Biotechnol 150(1):310

Urtubia A, Emparan M, Roman C, Hernández G, Roger JM (2010b) Multivariate statistic and pattern recognition to detect abnormal fermentations in wine process. J Biotechnol 150(1):328

Urtubia A, Hernández G, Román C (2011) Prediction of problematic wine fermentations using artificial neural networks. Bioprocess Biosyst Eng 34:1057–1065

Urtubia A, Hernández G, Roger JM (2012) Detection of abnormal fermentations in wine process by multivariate statistics and pattern recognition techniques. J Biotechnol 159:336–341

Varela C, Pizarro F, Agosin E (2004) Biomass content govern fermentation rate in nitrogen-deficient wine musts. Appl Environ Microbiol 70(6):3392–3400

Wu Z et al (2015) Monitoring of fermentation process parameters of Chinese rice wine using attenuated total reflectance mid-infrared spectroscopy. Food Control 50:405–412

www.techniquesinhomewinemaking.com

Yang Y, He Q, Hu X (2012) A compact neural network for training, support vector machines. Neurocomputing 86:193–198

Yu HY, Niu XY, Lin HJ, Ying YB, Li BB, Pan XX (2009) A feasibility study on on-line determination of rice wine composition by Vis-NIR spectroscopy and least-squares support vector machines. Food Chem 113(1):291–296

Index

S. K. Panda, P. H. Shetty (eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*, Food Microbiology and Food Safety,
https://doi.org/10.1007/978-3-319-74820-7