Chapter 13
Microbial Fermentation in Food Preservation

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Abstract Fermented foods are consumed worldwide representing a significant component of the human diet. Foods preserved by fermentation are perceived as a natural and healthy choice. The safe and palatable fermentation of foods is subjected to basic principles of acidification, salting, water activity and oxygen availability. Food fermentations are dependent on the activity of the type of microbes added as starter cultures. The multiple microbially mediated metabolic conversions in food fermentations impact bioactivity, stability, antimicrobial activity and toxicity of the finished product. This book chapter discusses basic preservation principles, microbial activity and chemistry of food fermentations.

Keywords Fermented foods • Lactic acid bacteria • Yeasts • pH • Preservatives • Antimicrobials

1 Description of Food Preservation by Fermentation

Fermentation is mainly regarded as a practical and effective mean to preserve foods with unique organoleptic properties, and minimal energy inputs (Daeschel et al. 1987; Leroy and De Vuyst 2004). It has been used to prevent the spoilage of raw foods since the Neolithic period (Bourdichon et al. 2012). While the discovery of fermentation is associated with production of alcoholic beverages and bread in Babylon and Egypt,

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today it represents a cosmopolitan industry with a billionary value in US dollars (Scott and Sullivan 2008; Konings et al. 2000). Although, extremely beneficial to human kind, the fermentation process remained largely uncharacterized for centuries. Deprived of an understanding of the microbiology behind complete and desirable fermentations, old generations use high quality cover brines or yeasts paste from fermentations with desirable attributes to initiate fresh ones, a technique known as back slopping. With the observations made by Anton van Leeuwenhoek in 1680 of living cells, using an early version of the microscope, and those contributed by Cagnard-Latour in 1839, fermentation was understood as a microbially induced process in which yeasts produce ethanol and carbon dioxide from sugars (Nanninga 2010).

An industrialist in Lille, France working with Louis Pasteur, discovered the role of lactic acid bacteria in fermentations. The problem of a reduced concentration of alcohol and sourness in ethanol production existed. Nevertheless, the discovery permanently changed the fermentation field. Pasteur published several papers between 1857 and 1860 documenting the replacement of the ethanol producing yeast population by microbes able to produce lactic acid in the fermenting samples. Such documentation was the first proof of the bacterial nature of fermentation, understood as a chemical degradation of sugars prior to the 1830s (Nanninga 2010). The very first pure starter culture was prepared by Joseph Lister in 1873 by diluting fermented milk. Realizing the potential impact of pure cultures in fermentations, 15 years later, Vilhelm Storch prepared pure cultures used for souring pasteurized cream (Knudsen 1931). The use of starter cultures for dairy fermentations was introduced around the 1890 in Copenhagen (Stiles and Holzapfel 1997). Defined fermentative cultures were introduced commercially in New Zealand in 1934 (Cogan and Hill 1993) beginning the era of "controlled" fermentations. Today a starter culture is defined as a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material to accelerate and dictate the course of a food fermentation (Leroy and De Vuyst 2004; Ayhan et al. 2005). The modern understanding of fermented foodstuff is thus the microbial metabolic process that converts sugars to acids, gases or alcohol to achieve long term preservation while generating desirable organoleptic properties. It is estimated that today 600,000 tons of the baker's yeast are sold annually (Pretorius et al. 2015). The total commercial production of starter cultures for large scale fermentation is estimated to be above 40,000 L annually used to inoculate tens of thousands of tons of raw material (Hansen et al. 2015).

2 Natural Acidification and Production of Preservatives in Fermented Foods

In food fermentations, organic substrates, usually a carbohydrate, is incompletely oxidized, and an organic carbohydrate acts as the electron acceptor with the release of energy (Sahlin 1999; Chacko et al. 2010; Chojnacka 2010). Two main reactions occur in food fermentations: conversion of sugars to ethanol and carbon dioxide by yeasts and/or conversion of sugars to organic acids and carbon dioxide by lactic acid bacteria. Homofermentative lactic acid bacteria convert one molecule of glucose to

two molecules of lactic acid. Heterofermentative lactic acid bacteria produce one molecule of lactic and acetic acids and one molecule of carbon dioxide per molecule of glucose. Conversion of sugars to organic acids generates a decrease in the pH of the food matrix beneficial for preservation.

pH, defined as the negative value of the logarithmic concentration of hydrogen ions, is a critical factor in controlling the quality and safety of food fermentations. High hydrogen concentration or an acidic pH is effective in protein hydrolysis and denaturation, which impairs the functionality of cell proteins and the microbial metabolic machinery (Meng et al. 2007), and impacts the physical state and texture of foods. The intracellular pH of most microorganisms is maintained near neutrality. Thus, an acidic pH may kill microbes intolerant to high hydrogen ion concentrations. However, fermentative lactic acid bacteria can tolerate pH values as acidic as pH 3.3 (McDonald et al. 1990). Few microorganisms can proliferate at pH values below 4.6, which is a critical control point in the production of acidified foods in the USA. If oxygen is present, most yeasts can proliferate at pH values between 4.5 and 6.0 at the expense of sugars or organic acids. Utilization of organic acids by yeasts or spoilage lactic acid bacteria results in an undesired increase in pH (Ruiz-Cruz and Gonzalez-Cancho 1969; Franco and Pérez-Díaz 2012).

Besides inducing a decrease in pH, the production of organic acids also increases the antimicrobial activity of fermented foods. Lactic acid is well known to reduce the populations of pathogenic microorganisms in fermented foods (Sahlin 1999). Organic acids are more effective at controlling microbial growth as compared to inorganic acids, partially due to the contribution of the undissociated species. The undissociated species of organic acids diffuse through the cell membrane and dissociates intracellularly resulting in: (1) cytoplasmic acidification and accumulation of anionic species (Booth and Kroll 1989), (2) the disruption of pH homeostasis and cellular metabolism (Beales 2004), and (3) the impairment of the proton motive force and active transport (Sheu et al. 1972). Although, different rankings for the effectiveness of organic acids have been published under a variety of conditions, it has been observed that lactic acid is more effective at reducing pathogens of public health significance under comparable conditions of pH, temperature, ionic strength and anaerobiosis (Lu et al. 2011). Preservatives commonly used in acidified foods such as sorbic acid, benzoic acid and fumaric acid are significantly more effective against pathogens, as compared to lactic acid (Lu et al. 2011). The effectiveness of organic acids in preservation is dependent on pH as determined by their dissociation constant. At neutral pH most organic acid exist in their dissociated forms, meaning that they release the hydrogen ion from their carboxilic groups. At acidic pH however, most of the organic acids exists in their undissociated forms or associated with their corresponding hydrogen ions. For instance, only 1.6% of the total amount of benzoic acid is present in its active form at pH 6.0. The dissociation constant for benzoic acid is 4.2. Thus, at pH 3.5 about 83% of the total acid concentration is active. The undissociated hydrophobic form of the organic acids diffuse over the cell membrane, dissociates inside the cell, and release H^+ ions that acidify the cytoplasm. In essence the undissociated form of the acids induces the collapse of the electrochemical proton gradient, causing bacterial death (Gürakan 2007). Different organic acids are inherently more or less effective against

specific microbes. In general terms, sorbic acid is more effective at controlling yeasts than benzoic acid, however lactic acid bacteria are insensitive to sorbic acid. Thus, sorbic acid is often incorporated in cover brines used for cucumber fermentation in which lactic acid is the desired metabolic product.

Lactic acid bacteria often outcompete yeasts in vegetable fermentations given their ability to grow and metabolize energy sources at a rate faster than spoilage yeasts and to produce antifungal compounds including organic acids, hydrogen peroxide, carbon dioxide, diacetyl, acetaldehyde, low molecular weight antmicrobial substances such as reuterin, 2-pyrolidone-5-carboxylic acid, bacteriocins, adhesion inhibitors, fatty acids, hydroxylated fatty acids, phenyllactic acid, and cyclic dipeptides (Gürakan 2007; Ganzle 2009). Hydrogen peroxide (H_2O_2) is an antimicrobial compound produced by most lactic acid bacteria by mean of flavoprotein containing oxidases, NADH oxidases, and superoxide dismutases in the presence of oxygen. Formation of hydroxyl radicals from hydrogen peroxide has long being considered as one of the main antimicrobial factors in fermentations. However, with the understanding that phytochemicals and antioxidants may formed during fermentations, in particular of plant derived foods, the impact of such compounds as antimicrobials is uncertain. Diacetyl, the major aroma and flavor component of butter, is a metabolic product of heterofermentation by some lactic acid bacteria. It has been documented that diacetyl has a bactericidal effect on strains of *Yersinia enterocolitica*, *Aeromonas hyrophilia*, *Escherichia coli* and *Salmonella anatum* (Naidu et al. 1999). Some *Lactobacillus* strains are capable of producing reuterin which consists of an equilibrated mixture of monomeric, hydrated monomeric and cyclic dimeric forms of 3-HPA (3-hydroxypicolinic acid). Reuterin is produced by *Lactobacillus reuteri* from glycerol by starving cells under anaerobic conditions (Montiel et al. 2014).

Although lactic acid bacteria are able to produce many antimicrobial compounds, bacteriocins are the most studied and commercially exploited to date (Leroy et al. 2003; Gürakan 2007; Altuntas et al. 2010; Altuntas et al. 2014). Even though bacteriocins or antimicrobial peptides were originally studied as a product of the Gram-negative *Escherichia coli*, the homolog produced by Gram-positive lactic acid bacteria LAB are of particular interest for commercial applications given the "Generally Recognized as Safe" status of such fermentative microbes (Fujita et al. 2007; Galvez et al. 2007; Liu et al. 2011; Cleveland et al. 2001; Deegan et al. 2006). The molecular weight of the many bacteriocins produced by lactic acid bacteria is below 10 KDa. Although the producing microbes are immune to the antimicrobial activity of such peptides, they are effective against other Gram-positive bacteria (Schillinger et al. 1996). Bacteriocins are considered natural antimicrobial compounds, which can be used as food additives, incorporated in fermented products using strains able to produce them, as starter cultures or by using a fermented food prepared with a bacteriocin producing starter culture as an ingredient (De Vuyst and Leroy 2007; Deegan et al. 2006). The use of purified bacteriocins is not always attractive to the food industry given that such peptides may be degraded by proteolytic enzymes in the stomach. However, bacteriocin producing probiotic strains may produce the peptides in the gastrointestinal tract and aid in the control of pathogens. Bacteriocinogenic lactic acid bacteria strains can be used as starter cultures, co-

cultures, or bioprotective cultures, to improve food safety. Bacteriocin producing starter cultures may be applied in the making of sourdough, fermented sausages, and cheeses to increase competitiveness, inhibit *Listeria* spp., and inhibit *Listeria* and *Clostridium* spp., respectively (De Vuyst and Leroy 2007; Cosansu et al. 2010).

Anaerobic metabolism of *Saccaharomyces* spp. is the primary source of ethanol production from sugars in alcoholic fermentations. Spoilage of alcoholic fermentations by lactic acid bacteria is likely the most common problem in achieving a high yield, due to the tolerance of the former to ethanol. Although, lactic acid bacteria can tolerate ethanol, and some produce it under certain conditions, the alcohol has the ability to impair the physiology of lactic acid bacteria. *Oenococcus oeni*, is one of the most resistant lactic acid bactetria to ethanol. The ability of *O. oeni* to resist up to 10% ethanol has been attributed to the ability to biosynthesize phospholipids and L-lactic acid to control membrane fluidity, metabolize citric acid and aggregate (Teixera et al. 2002; Olguín et al. 2009; Elahwany 2012). Ethanol concentration effective against non-lactic acid bacteria has been found to be around 5% (Oh and Marshall 1993), which is half of that concentration effective against *O. oeni*.

3 Relevant Extrinsic Parameters in the Production of Safe Biopreserved Foods

The safe biopreservation of foods is achieved by controlling the natural microbiota with starter cultures, salt, water activity, heat treatments, preservatives or the use of protective cultures (Devlieghere et al. 2004) and assuring the complete removal of primary sugars. Although harder to control in large scale fermentations, temperature is also a critical factor to achieve the desired fermentation.

Salt, primarily NaCl, influences the type and extent of microbial activity, helps prevent softening of food tissues, determines the flavor of the final product, and assists in rupturing the membranes of vegetables and fruits to be fermented, allowing the diffusion of various components into the cover brine solutions used by microbes for growth and metabolic activities (Pérez-Díaz et al. 2015 and references therein). In some foods high salt concentrations significantly retard or preclude fermentation (Pérez-Díaz et al. 2015 and references therein). Softening of vegetable tissues can be reduced or prevented by adjusting the salt level to retard the activity of pectinolytic enzymes derived from eukarotic cells (Bell and Etchells 1961; Bell et al. 1950).

Water activity is a measurement of the vapor pressure in a substance over that of pure water, essentially describing whether the conditions in a fermented product are conducive to microbial growth, primarily yeasts. Although, most brined fermented products are not susceptible to changes in water activity, it is a critical control point in fermented meat products. Adjustment in water activity in fermented meats is achieved by salting and drying. The higher the salt concentration the lower the water activity. Although, low water activity prevents microbial growth, it does not exert a killing effect and many microbes do not tolerate levels of salt lower than those

needed to achieve a low water activity. In sweetened fermented products, water activity is controlled by the addition of sugars. Bacteria are more sensitive to sugars as compared to yeasts, some of which can proliferate in saturated sugar concentrations. Yeast species commonly found in fermented vegetables, meats and alcoholic beverages such as *Candida*, *Torulopsis*, and *Hansenula* can grow at water activity of 0.87 or higher. *Saccharomyces* and *Debaryomyces* species can tolerate a water activity as low as 0.80 (Beuchat 1983). Significantly higher concentrations of sugars are needed to lower water activity as compared to salts. For instance, 19% sodium chloride is needed to drop the water activity to 0.85 at 25 °C. More than 60% glucose is needed to achieve a water activity of 0.85 under comparable conditions.

The microbiological nature of fermentation imposes a temperature effect to achieve preservation (Rodgers 2001; Kaur et al. 2011). Generally, lower temperatures reduce the movement of molecules and frequently prolongs the fermentative microbes doubling time. Although, fermentative lactic acid bacteria are mesophilic and capable of growing at temperatures between 5 and 45 °C, their optimum growth temperature is between 30 and 40 °C (Caplice and Fitzgerald 1999). Although, optimum growth temperature assist in achieving fast fermentation, it can also influence the quick initiation of spoilage by undesired microbes. Thus, often times a variety of extrinsic factors are used in food fermentations, to incorporate the necessary hurdles for growth of non-desired microbes, while creating the best conditions for the proliferation of the desired fermentative bacteria and/or yeasts. Optimum growth temperature for yeasts varies between −2 and 45 °C (Arthur and Watson 1976), however, one of the most studied fermentative yeast essential in winemaking, baking and brewing, *Saccharomyces cerevisiae* grows optimally between 30 and 35 °C (Walsh and Martin 1977). While yeast cells are more sensitive to heating treatments as compared to bacteria, they survive freezing under certain conditions, with viability decreasing over time (Arthur and Watson 1976). Short time and relatively low temperature (70 °C) heating steps are often used on fresh produce prior to fermentation to induced inactivation of softening enzymes, release simple sugars from polyssacharides or starch, and reduced the initial microbial load. This is particularly relevant in the processing of vegetables, tubers and fruits.

Fermentation is regarded as an anaerobic metabolic process, meaning that oxygen is not necessary for the conversion of sugars to organic acids and/or alcohol. Lactic acid bacteria are facultative anaerobic, meaning that they do not have a requirement for oxygen to grow but can tolerate it. Acetic acid bacteria cannot grow in the absence of oxygen. *Saccahromyces cerevisiae* may grow and ferment under aerobic or anaerobic conditions, however most yeasts have an oxygen requirement. Fermentative yeasts can grow well in the absence of oxygen, if sugars are available. Control of oxygen availability in a fermentation process is critical for the achivement of the desired microbial metabolism and the inhibition of spoilage and pathogenic microbes. In most fermentations the appropriate combination of factors that can affect microbial growth results in safe and high quality products. The combination of pH below 4.6 and anaerobiosis, for instance, prevents the proliferation of *Clostridium botulinum* and corresponding toxin production, which may occur should the pH remained above 4.6.

4 Physical and Nutritional Changes in Fermented Foods

Aside from preservation, microbial fermentation detoxifies foods, improves accessibility of nutrients, and improves organoleptic properties (Bourdichon et al. 2012; Caplice and Fitzgerald 1999; Liu et al. 2011). During a fermentation process, a significant increase in the soluble fraction of a food is observed (Sahlin 1999). The quantity as well as the quality of the water soluble vitamins and the food proteins, expressed as the biological value, increases, while the antinutritional factors show a decline as the result of fermentation (Sahlin 1999). Yoghurt in particular contains almost all nutrients present in milk in a more assimilable form (Chacko et al. 2010). The toxic cyanide content of cassava is reduce during the fermentation and acidification of the tuber by linamarase (Ikediobi and Onyike 1982). Milk digestibility is increased in cheeses with a reduced lactose content. The phytochemical content and antioxidant properties of fermented plant derived foods increase as compared to the fresh vegetables and fruits (Yeo and Ewe 2015). The antioxidant content contributes to the health benefits of plant foods by reducing the incidence of age-related diseases such as heart conditions and some cancer types (Yeo and Ewe 2015).

Although the development of characteristic flavors is a critical factor in fermented foods, specific mechanisms to achieve the desired flavor remain unknown (Caplice and Fitzgerald 1999). Lactic acid bacteria have a significant role on the composition of non-volatile and volatile compounds through the release and/or degradation of free amino acids and prevent the oxidation of unsaturated free fatty acids associated with rancidity (Fadda et al. 2010).

The microbial activity in fermented foods also results in an increment in the water soluble exopolysaccharides, conjugated linoleic acid, and bioactive peptides content. Exopolysaccarides or long sugar chains occasionally combined with phosphate, acetyl, uronic acid and glycerol (Nwodo et al. 2012) are produced by microbial metabolism utilizing the fermenting food matrix components (De Vuyst and Degeest 1999). The proteolytic activity of lactic acid bacteria present in fermented foods, primarily in dairy products, generates peptides that are further modified in the gastrointestinal tract (Pihlanto and Korhonen 2015). Conjugated linoleic acid isomers are derived from rumminants and consequently found in fermented meats and dairy products (Csápo and Vargas-Visi 2015). Conjugated linoleic acid isomers are generally recognized as safe by the US Food and Drug Administration and may be produced by fermentative lactic acid bacteria under certain conditions. These metabolically produced compunds have been associated with beneficial health effects and the improvement of organoleptic properties in fermented products. Exopolysaccharides are used in finished yogurt and bread products as texturizers, viscosifiers, emulsifiers and synerisis-lowering agents given their plasticity and water absorbing capacity (Cerning 1990). Proteolysis and peptidic systems have been implicated in the texture, taste and flavor of fermented dairy foods. Multiple reviews can be found in the literature regarding the potential functionality of bioactive peptides, exopolysacharides and conjugated linoleic acids as immunomodulators, cytomodulators, anticholesterole, oxidants, microbial, cancer, tumors, ulcers and genetic mutations, protectants of the intestine, anti-diabetes, obesity and cardiovascular diseases, and mineral binders (Duboc and Mollet 2001; Jolly et al. 2002; Ruas-Madiedo et al. 2002; Aswathy et al. 2008; Ruas-Madiedo et al. 2006; Csápo and Vargas-Visi 2015).

5 Types of Fermented Foods and Interactions of the Microorganisms Involved

Desirable fermentations occur when organisms able to preserved a food are capable of outcompeting the microbiota naturally present in the substrate. Fermentative organisms are intrinsic in the environment, thus production of fermented foods does not require knowledge of the biologically mediated nature of fermentation (Scott and Sullivan 2008). Human survival is connected to yeasts and bacteria that produce lactic acid and alcohol in preserved foods (Scott and Sullivan 2008). Fermentative lactic acid bacteria and yeasts are capable of competing for nutrients in foods, such as the sugars, nitrogen sources, and vitamins. Robust fermentative lactic acid bacteria and yeasts create unfavorable conditions, such as an acidic pH or high ethanol concentrations to exclude competing microbes from the food matrix. Fermentative microbes also adhere or localize near the sources of nutrients (Caplice and Fitzgerald 1999).

Fermented foods could be classified as dairy, starchy foods, cereals, meat, vegetables, alcoholic beverages, and legumes and oil seeds (Caplice and Fitzgerald 1999; Achi 2005). Table 13.1 describes the main types of fermented foods and microorganisms involved. In a significant proportion of fermented foods, *Enterobactereaceae* initiate the fermentation until lactic acid bacteria produce enough lactic acid to reduce the pH of the food matrix. In some food fermentations, such as cocoa, acetic acid bacteria convert the product of primary fermentation into acetic acid by means of membrane bound or cytoplasmic dehydrogenases (Scott and Sullivan 2008).

As mentioned above, lactic acid bacteria are responsible for many of the microbial transformations found in popular acidic fermented food products (Ross et al. 2002). Lactic acid bacteria consists of 12 genera, among them; *Lactococcus*, *Lactobacillu*s, *Leuconostoc*, *Enterococcus*, *Streptococcus* and *Bifidobacterium* are the most commonly studied and used in commercial applications (Özer 2007). Lactic acid bacteria are Gram-positive, catalase and oxidase negative, facultative anaerobic rods and cocci, which generally have complex nutritional requirements especially for amino acids, vitamins, and purines. This group of bacteria have a number of auxotrophies and weak lipolytic activity, which defines their need to dominate in food matrixes (Caplice and Fitzgerald 1999; Françoise 2010). While the main catabolite of homofermentative lactic acid bacteria from sugars is lactic acid; heterofermentative lactic acid bacteria convert sugars to lactic acid, acetic acid and carbon dioxide.

Even though, enterococci, lactic acid bacteria able to colonize milk and meat, are considered by some as unacceptable for food production due to their ability to colonize the instestinal tract (Khan et al. 2010). Given its ability to colonize raw meats, *E. faecium* is frequently found in meat fermentation in significant numbers contributing to lactic acid production and the inhibition of *Listeria monocytogenes*, and the sensory characteristics resulting from the ripening of cheese. It is understood that *E. faecium* metabolic ability includes lipolysis, citric acid utilization, and the production of aromatic volatile compounds (Hugas et al. 2003; Leroy et al. 2003; Gürakan 2007; Giraffa 2003). Bacteriocin production by *E. faecium* RZS C5 under acidic conditions during logarithmic phase, as opposed to stationary phase, makes its use as

Table 13.1 Description of the primary microbiota in selected fermented foods

Fermented food	Particularities of the process or microbes	Participating microbes	Review reference
Sausage	Psychrophilic lactobacilli-pentose utilizers	*Lactobacillus sakei*, *L. curvatus*	Lücke (2015)
	Catalase positive cocci-superficial fermentation	*Staphylococcus warneri, S. xylosus, S. equorum, S. succinus, S. carnosus*	
	Unsmoked, superficial ripening	*Penicillium nalgiovense, Debaryomyces hansenii*	
Fish	Halophilic LAB (>10%)	*Tetragenococcus* spp.	Kuda (2015)
		L. plantarum, L. rennini, L. acidipiscis, L. versmoldensis	
		Debaromyces hansenii,Pichia anomala and superficial molds	
Bread		*baker's yeast*	Brandt (2015)
	Sourdough	*L.sanfranciscensis, L. reuteri, L. pontis, L. crispatus, L. paralimentarius*	
Soy	Inoculated	*Aspergillus oryzae*	Nout (2015)
	Halophilic microbes (~20%)	*Tetragenococcus halophilus, Zygosaccharomyces rouxii*	
	Doujiang	*Bacillus* spp., *L. fermentum*, *L. plantarum*	
		Candida humilis, Kluyveromyces lactis, Williopsis saturnus, Sac. gallinarum	
Wine	Primary sugar utilization	*Sac.cerevisiae*	Bisson and Walker (2015)
	Proton motive force as source of energy	*Oenococcus oeni, L. hilgardii, L. brevis, L. plantarum, L. pentosus*	
	Utilization of organic substrates	Acetic Acid Bacteria	
	Ripening	*Aureobassidium, Cryptococcus, Rhodosporidium*, and *Rhodotorula*	
Beer		*Sac. pastorianus* (formerly *S. carlsbergensis*)	Kyselová and Bránayik (2015)
	Ale yeasts	*Sac. cerevisiae*	
	Spoilage	*Megasphaera* and *Pectinatus* spp.	
Coffee	Initiation of the fermentation	*Enterobacteriaceae*	Huch and Franz (2015)
	Undefined and highly variable	Bacteria, yeasts and filamentous fungi	

(continued)

Table 13.1 (continued)

Fermented food	Particularities of the process or microbes	Participating microbes	Review reference
Vegetables	Initiation of the fermentation	*Enterobacteriaceae*	Medina-Pradas et al. (2016)
	Active fermentation	*L. plantarum, L. pentosus, L. brevis, Pediococcus* spp., *Leu. mesenteroides, Weisella* spp.	
		Candida spp., *Pichia* spp. and *Saccharomyces* spp.	
	Scum formation	*Debaryomyces* spp., *Pichia* spp., *Mycoderma*	
Dairy		Various subspecies of *Lactococcus lactis* and *L. delbrueckii*	Leroy and De Vuyst (2004)
		Leu. mesenteroides subsp. *cremoris*, *Streptococcus thermophilus*	

an adjunct culture advantageous (Leroy et al. 2003). Selected *Enterococcus spp.*, are used as starter cultures in dairy products and widely used as probiotics (Khan et al. 2010).

The ability of lactic acid bacteria to produce exopolysaccharides aids them to survive under stressful conditions, including the acidic pH, and high salt (Cerning et al. 1994; Donot et al. 2012; Harutoshi 2013) characteristic of fermented foods. It also aids in resisting bacteriophage infections and the antimicrobial effect of preservatives and toxic ions, in establishing their ecological niche through cell adhesion, biofilm formation and quorum sensing (Ruas-Madiedo et al. 2002; Kodali et al. 2009; Nwodo et al. 2012), and in nutrient accumulation (Patel and Prajapati 2013). Exopolysaccahrides also help the producing bacteria to outcompete the natural microbiota and survive in extreme habitats such as those with high or cold temperatures (Nichols et al. 2005; Kim and Yim 2007; Poli et al. 2010).

Ocassionally the lactic acid produced during fermentation can be utilized as an energy source by spoilage organisms such as *Propionebacterium* spp., *Pichia manshurica*, *Issatchenkia occidentalis*, *Lactobacillus buchneri*, *Lactobacillus rapi* and *Lactobacillus namurensis* (Gonzalez-Cancho et al. 1970, 1980; Franco et al. 2012; Medina-Pradas et al. 2016). Removal of the acid from the fermentation matrix causes an increase in pH, which enables the growth of other spoilage and pathogenic microbes. Thus, extrinsic factors should be appropriately applied to achieve the stable fermentation of foods.

Competing fermentative lactic acid bacteria strains can be selected based on their ability to grow in the food matrix of interest and outcompete and survive selected pathogens of public health significance. *Lactococcus lactis* subsp. *lactis* and *Pediococcus acidilactici* are used as biocontrol for pathogens like *Salmonella enterica*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in alfalfa seeds and sprouts (Kostrzynska and Bachand 2006; Altuntas 2013).

The baker's yeast, *Saccharomyces cerevisiae,* is well known today for being the first to be observed under the microscope, and the first to be recognized as a living organism able to biochemically transform bread and alcoholic beverages (Pretorius et al. 2015). Even though it is the most frequently used yeast for fermentations; it is assisted by *O. oeni* for the conversion of malic acid into lactic acid in wine making. The lactic acid bacterium transforms the sharp apple like flavor of malic acid to the softer tasting lactic acid (Pretorius et al. 2015). The co-existence of the baker's yeast with *O. oeni* in wines is possible due to the resistance of the former to more than 10% ethanol (Teixera et al. 2002; Olguín et al. 2009; Elahwany 2012). However, the lactic acid bacteria may cause spoilage at different stages of wine making and its proliferation has to be monitored and controlled to retain quality.

6 Advantages of the Utilization of Authoctonus Starter Cultures and Probiotic Cultures for Food Fermentations

Development of starter and adjunct cultures for food fermentations represents a field heavily researched since the 1930s. Such studies have been recently accelerated by the advances in high-throughput sequencing technology, deepening insight into complex fermentation systems (Bokulich and Mills 2012; Ivey et al. 2013). Although, to date the traditional fermentation processes rely on the natural microbiota and their spontaneous proliferation in foodstuffs, the industrial production of fermented foods heavily relies on the use of starter cultures (De Vuyst and Leroy 2007). Utilization of selected starter culture aids in accelerating the initiation of fermentation, shortening of the fermentation process and reduction of the risk of fermentation failure (Leroy and De Vuyst 2004; Ivey et al. 2013). It has been demonstrated that food fermentations with autochthonous starter cultures are efficient and produced high quality finished products as compared to allochthonous or wild fermentation (Di Cagno et al. 2008a, b, 2009). More specifically, a faster reduction in pH and proliferation of desired microbes proceeds in vegetables fermented with autochthonous starter cultures. Longer lag phases have been observed for allochthonous starter cultures (Di Cagno et al. 2008a, b, 2009). Quality and nutritional attributes such as color, sensory and rheological properties, vitamins and antioxidant activity of fermented vegetables are favorably impacted and retained when autochthonous starter cultures are used (Di Cagno et al. 2008a, b, 2009, 2013). Autochthonous bacteria have an intrinsic ability to produce metabolites such as bacteriocins and exopolysaccharides, that are tailored specifically for their establishment during fermentation, and thus have a greater chance of survival when used as starter cultures (Di Cagno et al. 2013). Selected secondary metabolites specifically produced by allochthonous starter cultures in their native food matrix, such as bacteriocins, exopolysaccharides and vitamins, are often times considered added value to the final product (Di Cagno et al. 2009).

Most of the commercialized starter cultures include the group of lactic acid bacteria and the bread making yeast *Saccahromyces* spp. Aside from serving as starter cultures,

Bifidobacterium, *Lactobacillus* and to a lesser extend *Saccahromyces* species also serve as supplementary probiotic cultures. Probiotic cultures are generally defined by the Food and Agriculture Organization-World Health Organization (WHO) as live microbes that when added in foods confer a beneficial health effect to the host (WHO 2002; Morelli and Capurso 2012) and are generally added to 10^9 colony forming unit per serving size (Forssten et al. 2011). Although most fermented foods provide for the growth of probiotic cultures, only dairy and soy derived products have been labeled to contain probiotic cultures, mainly due to the compatibility of processing with the survival of the microbes to the required levels (Ouwehand and Röytiö 2015). Probiotic cultures are not generally effective fermenters and can benefit from the presence of robust compatible starter cultures in fermented foods. Although, the most recognize health effect from probiotic cultures is in the recovery of antibiotic treated diarrhea, they have also been implicated in the exclusion of pathogens from the gastrointestinal tract, shortening of colonic transit, modulation of the host immunity, and improvement of metabolism (see Ouwehand and Röytiö 2015 for a review; Gürakan 2007).

7 New Trends in Food Fermentations

Traditional spontaneous fermentation will continue to be central to the production, availability and supply of foods for a growing population. Current consumer trends are aligned with the deliberables of fermented foods, such as increased bioavailability and bioactivity of natural components and the demand for natural processes to create healthy foods with reduced environmental impact. Additionally, food fermentations done with standard hygienic practices and the recommended processes very often result in safe food alternative for consumers. In a decade when, non-thermal preservation technologies such as high hydrostatic pressure and pulsed electric fields, and new packaging systems with modified atmospheres are subjected to scrutiny by regulatory agencies and offer limited production capacity, food fermentation will continue to deliver.

Food fermentations in which starter cultures are added are likely to continue to emerge as alternatives with enhanced nutrition and flavor. The use of molecular genetic tools is expected to result in bacteriocinogenic cultures that combined with other food additives or technologies could offer stable and savory products (Devlieghere et al. 2004; Settanni and Corsetti 2008).

Enhanced packaging and sanitizers for food contact surfaces are under development and expected to result in a generation of polymers and antimicrobials that could enhance the long term stability of fermented foods (Appendini and Hotchkiss 2002).

The selection, design and development of starter cultures for fermented foods will continue to be the main research focus in this field, more so with the availability of new molecular genetic techniques, the multiple-omics technologies and bioinformatics. Starter cultures capable of positively impacting the organoleptic properties of foods, reduce the toxic content of foods or modify the undesired microbial activ-

ity in the gut, and contribute to the health of consumers will continue to be desirable (van Hylckama Vlieg et al. 2011).

Advances in genomics and physiology of lactic acid bacteria are enabling a link between individuals genetic background and metabolic traits of starter cultures to specific food quality attributes (Ganzle 2009). Selection of starter cultures will continue to be increasingly influenced by their functionality. Development of optimized food fermentation systems with optimal starter cultures and chemical composition is around the corner.

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