CHAPTER

YOGURT-LIKE BEVERAGES MADE WITH CEREALS

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10.1 NONDAIRY BEVERAGES, THE FUTURE OF FUNCTIONAL FOODS

Over the last decade, the demand for "healthy" foods and beverages has increased on a global scale, and the diffusion of functional foods throughout the market overlapped the interests of the pharmaceutical and food sectors (Corbo et al., 2014). Nowadays, the advances in scientific research support the idea that diet may fulfill nutritional needs and exert a beneficial role in some diseases (Otles and Cagindi, 2012). Several critical factors have been recognized as the key factors leading to the diffusion of functional foods: health deterioration, due to busy lifestyles, consumption of convenience foods, and insufficient exercise; increased incidence of self-medication; increased awareness of the link between diet and health due to information by health authorities and media on nutrition; and a crowded and competitive food market (Granato et al., 2010). Above all, the various stakeholders have perceived the economic potential of functional food products as an important part of public health prevention strategies. According to the recent literature and legislation, functional foods can be defined as "foods and food components that provide a health benefit beyond basic nutrition" (Serafini et al., 2012), and in particular as a "food similar in appearance to, or may be, a conventional food that is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions" (Lau et al., 2013). According to these definitions, foods in which a component has been modified by enzymatic, chemical, or biotechnological means to provide a benefit are included (Pravst, 2012).

Among the large number of novel and innovative functional foods under investigation or already present in the market, beverages are considered the most promising category because of (1) convenience and possibility to meet consumer demands for container contents, size, shape, and appearance; (2) ease of distribution and better storage for refrigerated and shelf-stable products; and (3) great opportunity to incorporate desirable nutrients and bioactive compounds (Corbo et al., 2014). Several different types of commercial functional beverages are today available (e.g., dairy-based beverages including probiotics and minerals/ω-3-enriched drinks, sports and energy drinks), and there is a growing interest toward nondairy beverages, made with vegetables, fruits, and cereals (Corbo et al., 2014; Granato et al., 2010; Kandylis et al., 2016).

Milk has been long considered as the only food containing all the essential substances for human nutrition. However, a study has recently reported that some milk constituents and common contaminants (such as pesticides, estrogen, and insulin-like growth factor 1) might be responsible for adverse reactions on the consumer's health (Davoodi et al., 2013). Moreover, lactose intolerance/lactose malabsorption and cholesterol content are major drawbacks related to functional dairy products (Prado et al., 2008). Up to 70% of the world population has lactase nonpersistence, although lactose tolerance

Yogurt in Health and Disease Prevention. http://dx.doi.org/10.1016/B978-0-12-805134-4.00010-9

could be affected by many nutritional and genetic factors. In Europe, the prevalence of lactose intolerance is about 5% in the British population and has increased to 17% in Finland and northern France (Lomer et al., 2008). The prevalence is above 50% in South America, Africa, and Asia, reaching almost 100% in some Asian countries (Zannini et al., 2013). In the United States, the prevalence is 15% among whites, 53% in Mexican Americans, and 80% in the African American population. Australia and New Zealand have a prevalence of lactose intolerance of 6%–9% (Tomar, 2014).

The health issues, in combination with the growing trend of vegetarianism and the limited use of dairy products in the diet of many countries, especially in Asia and Africa, has led to the development of nondairy beverages, designed as functional products also suitable for the delivery of probiotics (Granato et al., 2010). Furthermore, nondairy beverages are also considered as a more economical alternative to dairy products in developing countries. Nondairy beverages in the form of traditional products have long existed all over the world (such as boza, bushera, chhang, chica, haria, mahewu, omegisool, pozol, togwa) mainly based on cereals (Kandylis et al., 2016). In addition to these, several new nondairy probiotic beverages have been developed (Soccol et al., 2012). A huge potential has been recognized to using cereals as vehicles for probiotics and functional compounds such as antioxidants, dietary fiber, minerals, prebiotics, and vitamins (Nionelli et al., 2014); this is the reason why different commercial cereal-based beverages are today available on the market, such as Proviva (Skane Dairy, Sweden), the first oat-based probiotic food beverage produced using *Lactobacillus plantarum* 299v (Prado et al., 2008), and Whole Grain Probiotic Liquid (Grainfields, Australia), a refreshing, effervescent beverage containing both lactic acid bacteria (LAB) (*Lactobacillus acidophilus*, *Lactobacillus delbrueckii*) and yeasts (*Saccharomyces cerevisiae* var. *boulardii* and *S. cerevisiae*) as well as vitamins, amino acids, and enzymes (Soccol et al., 2012) (Table 10.1).

10.2 LACTIC ACID BACTERIA AS STARTERS FOR CEREAL-BASED BEVERAGES FERMENTATION

10.2.1 ADVANTAGES OF FERMENTATION

Fermentation has long been used as a way to naturally enhance the food matrix, without the need for additives or preservatives (Hugenholtz, 2013). In order to facilitate the control and reproducibility of final product qualities, the industry uses defined starter cultures. LAB have long been used as such in many food substrates, e.g., milk, meat, vegetables, and cereals, as well as being part of their indigenous microflora (Holzapfel, 1997), and many of them have been granted with the "generally recognized as safe" (GRAS) status. The genera *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and *Weissella* are naturally found on the surface of grains and in the surrounding environment (Guyot, 2012). For this reason they often form the natural inoculum, together with fungi, of fermented cereal gruels commonly consumed in many rural societies worldwide (Nout, 2009). Fermentation of cereal-based beverages by LAB has been shown: (1) to improve protein digestibility (Holzapfel, 1997; Peyer et al., 2016), due to the proteolysis occurring during fermentation (Coda et al., 2014; Nionelli et al., 2014); (2) increase nutritional bioavailability of minerals through the degradation of phytic acid by the activation of endogenous phytases and/or due to the contribution of microbial phytases (Coda et al., 2014; Nionelli et al., 2014); (3) increase the bioaccessibility of other nutrients (e.g., polyphenols, and fibers) (Coda et al., 2015; Nionelli et al., 2014); (4) decrease the glycemic index through the biological acidification and affect the rate of

Table 10.1 Traditional, Commercial, and Experimental Cereal-Based Fermented Beverages

Name	Cereal	Microorganism	References
Traditional			
Boza	Barley, oats, rye, millet, maize, wheat, rice	Lactic acid bacteria (LAB) and yeasts	Akpinar-Bayizit et al. (2010)
Bushera	Sorghum	LAB	Muyanja et al. (2003)
Chhang	Millet	LAB and yeasts	Thakur et al. (2015)
Chicha	Rice	LAB and *Bacillus* spp.	Puerari et al. (2015)
Haria	Rice	Yeasts, molds, LAB, *Bifidobacterium* spp.	Ghosh et al. (2014)
Mahewu	Maize, sorghum, millet	LAB	Mugochi et al. (2001)
Omegisool	Millet	LAB and yeasts	Kandylis et al. (2016)
Pozol	Maize	LAB, yeasts, molds	Ben Omar and Ampe (2000)
Togwa	Maize, sorghum, millet, cassava root	LAB and yeasts	Kitabatake et al. (2003)
Nigerian ogi	Maize, rice, sorghum, millet	LAB and yeasts	Adebayo-tayo and Onilude (2008)
Koko	Oat	LAB	Lei and Jakobsen (2004)
Kvass	Rye	LAB and yeasts	Jargin (2009)
Amazake	Rice	*Aspergillus* spp.	Yamamoto et al. (2011)
Experimental			
–	Emmer	*Lactobacillus plantarum*	Coda et al. (2011)
–	Malt	*Lactobacillus acidophilus*	Salmerón et al. (2014, 2015)
–	Rice, barley, emmer, oat, soy	*L. plantarum*	Coda et al. (2012)
Experimental tarhana	Wheat	*L. sanfranciscensis*, *L. plantarum*	Magala et al. (2014)
–	Oat	*Lactobacillus delbrueckii* subsp. *bulgaricus*	Grobben et al. (1997)
–	Oat	*Pediococcus damnosus* and yogurt starter	Mårtensson et al. (2002)
–	Emmer	*Weissella cibaria*	Coda et al. (2011)
–	Barley	*W. cibaria*	Zannini et al. (2012)
–	Oat, wheat, barley	*L. plantarum*	Salmerón et al. (2009)
–	Sorghum	–	Muyanja et al. (2012)
–	Oat	*Bifidobacterium* spp., LAB	O'Connor et al. (2005)
–	Oat bran	*Lactobacillus rhamnosus*	Loponen et al. (2007)
–	Whole-grain	*L. plantarum*	Loponen et al. (2007)
Oagurt	Oat	*L. acidophilus*, *Lactobacillus casei*, *Bifidobacterium*	Walsh et al. (2010)
–	Oat	*L. plantarum*	Nionelli et al. (2014)
–	Oat	*Lactobacillus reuteri*, *L. acidophilus*, *Bifidobacterium bifidum*	Mårtensson et al. (2001)

Continued

Table 10.1 Traditional, Commercial, and Experimental Cereal-Based Fermented Beverages—cont'd

Name	Cereal	Microorganism	References
–	Oat	*L. plantarum*	Angelov et al. (2005)
–	Oat	*L. plantarum*, *Lactobacillus paracasei* ssp. *casei*, *L. acidophilus*	Gokavi et al. (2005)
Commercial			
Proviva	Oat	*L. plantarum* 299v	Prado et al. (2008)
Whole grain probiotic liquid		*L. acidophilus*, *L. delbrueckii*, *Saccharomyces cerevisiae* var. *boulardii*, *S. cerevisiae*	Soccol et al. (2012)
Yosa	Oat	*L. acidophilus* and *B. bifidum*	Blandino et al. (2003)

All the traditional beverages are produced by spontaneous fermentation, without the addition of starters, and can be characterized by various structures (liquid to semiliquid texture), while all the experimental beverages reported in the table are characterized by a yogurt-like texture.

resistant starch (De Angelis et al., 2009); (5) prolong shelf life through the acidification and the release of antimicrobial compounds (Angelov et al., 2006; Gupta et al., 2010); and (6) enhance organoleptic quality (Nionelli et al., 2014; Peyer et al., 2016). The favorable macro- and micronutrients profile of cereals has made them an excellent candidate for functional food production through LAB fermentation (Blandino et al., 2003), providing the necessary environment for their growth while at the same increasing the bioaccessibility of these compounds. Bioaccessibility, which is the release of the compound from its natural matrix to be available for intestinal absorption, is in fact the first limiting factor to bioavailability (Endo and Dicks, 2014). The bioavailability of nutrients that are usually bound as reserve molecules in the form of starch and proteins can be enhanced with the addition of malted cereals, either directly or by adding to the pool of hydrolytic enzymes with, e.g., amylases, glucanases, and peptidases (Gupta et al., 2010; Nionelli et al., 2014).

10.2.2 FUNCTIONALITY

Besides acidification, LAB also have the ability to contribute to the synthesis and the release of several important nutraceuticals (Waters et al., 2015). The mechanisms by which LAB fulfill the role of efficient cell factory for the production of functional biomolecules were largely demonstrated for cereal-based beverages (Coda et al., 2011; Nionelli et al., 2014). Cereal fermentation leads to the decrease in the carbohydrates level as well as some not-digestible poly- and oligosaccharides, while the availability of certain amino acids and B vitamins is improved (Gobbetti et al., 2010). Furthermore, other compounds having biological functions such as gamma-amino butyric acid and biogenic peptides can be produced especially when selected LAB are used (Coda et al., 2010, 2012). Indeed, the selection of appropriate starter cultures for different kinds of cereal beverage is needed by the industry to drive, accelerate, and standardize the fermentation (Coda et al., 2014; Nionelli et al., 2014).

An important step to improve the nutritional value of fermented foods is through the activity of functional bacteria (Gobbetti et al., 2010) such as probiotics. The most important traits for a promising probiotic rely upon (1) survival in low pH and with bile salts added; (2) adhesion to intestinal epithelium; (3) antimicrobial activity toward foodborne pathogens and competitive adhesion to mucosa; (4) immunomodulation; (5) safety issues (production of harmful metabolites, like biogenic amines), and (6) transmission of genes encoding antibiotic resistance (Nagpal et al., 2012; Sip and Grajek, 2009). In addition to these characteristics, many LAB and *Bifidobacterium* spp. have been reported to produce vitamins such as folate, cobalamin, menaquinone (vitamin K), riboflavin, and thiamine. The use of these cultures in food fermentation potentially provides routes not only to enhance the nutritional profile of the food but also to deliver microorganisms to the gut, where they can synthesize such vitamins in vivo (O'Connor et al., 2005). It is commonly thought that the microbial colonization of the intestinal epithelium can give the best effect, since they can affect the intestinal immune system, displace enteric pathogens, provide antimutagens and antioxidants, and possibly other effects by cell signaling (Park and Floch, 2007). Apart from these classical properties, some additional features are required for probiotics in functional beverages, like the interaction with the starter cultures, as antagonistic interaction between probiotics and starter cultures may result in growth inhibition by acid, peroxide, bacteriocins, and other metabolites (Brajdes and Vizireanu, 2013; Nagpal et al., 2012). Similarly, the ability of probiotics to grow well in cereal or fruit and vegetable juices could depend, respectively, on their ability to exhibit amylolytic activity or to resist the effects of preservatives (Yeo et al., 2011).

Due to the fact that the prolongation of shelf-life is a great challenge for functional beverages, some researches have tried to improve the viability of probiotics. They should be maintained in the food product until the time of consumption and be present in significant numbers, at levels of at least 10^7 viable cells per gram or milliliter. Many approaches have been proposed, like a modification of the atmosphere of the product based on the increase of the content of CO_2 in the headspace, which might have an impact on the survival of microaerophilic and anaerobic bacteria (Walsh et al., 2014). Also, addition of ascorbic acid (vitamin C) might have a protective effect on probiotic cells during storage, presumably because it is an oxygen scavenger, thus promoting a more favorable anaerobic environment (Shah et al., 2010).

A further aspect related to the exploitation of microorganism functionality includes the production of bacterial polysaccharides. The in situ production of oligosaccharides and exopolysaccharides (EPSs) by *Weissella* spp. was previously reported also for different cereals (Kajala et al., 2016; Katina et al., 2009; Zannini et al., 2013). It has been suggested that the health-promoting effects of EPS-producing strains are related to the biological activities of these biopolymers. Exopolysaccharides might contribute to human health by affecting gastrointestinal functionality as prebiotics or due to antitumor, antiulcer, immunomodulating, or cholesterol-lowering activities (De Vuyst and Degeest, 1999). Some probiotic bacteria also are able to produce EPS and have been already employed for several fermented dairy products (Ruas-Madiedo et al., 2002). The ability to affect the texture by acting as emulsifiers or stabilizers is one of the most important features of EPS and is discussed later.

Finally, it should be emphasized that the health benefits imparted by probiotic microorganisms are strain specific, and that not even strains of the same species will be effective against defined health conditions or will provide all proposed benefits (Shah et al., 2010). As not all the desirable properties are expressed by all probiotic microorganisms, the screening and selection of potential probiotic starters is still important (Kumura et al., 2004; Ouwehand et al., 2002).

10.2.3 EFFECT ON TEXTURE AND SENSORY QUALITY

The ability of some LAB to excrete high-molecular-weight polysaccharides that can increase the viscosity of the liquid substrate is a very relevant feature in the production of cereal-based yogurt-like beverages. EPS are formed through polymerization of sugar subunits and can be either composed from repeating glucose or fructose subunits (homopolysaccharides) or from two or more different subunits (heteropolysaccharides) (Galle and Arendt, 2014). The type of EPS produced and its amount depends principally on the sugars present in the medium (Galle and Arendt, 2014), which can act as substrate or as acceptor molecules, on the presence of micronutrients (e.g., minerals acting as enzymes cofactors), and the environmental conditions of fermentation (e.g., incubation temperature and time) (Kajala et al., 2016).

The in situ production of EPS is of particular interest to manufacturers of fermented cereal-based drinks as they can resemble dairy products (Peyer et al., 2016). These products can therefore make a "natural," "additive free" claim and, at the same time, avoid the costs that result from the expensive and laborious EPS purification procedures (Badel et al., 2011). Dextran, for instance, a flavorless homopolysaccharide composed of glucose subunits, is a GRAS-granted thickener already used by the food industry (Peyer et al., 2016).

The ability of LAB to release texture-enhancing EPS has been found in many starter cultures involved in the production of traditional beverages. For instance, a significant number of LAB strains isolated from Nigerian ogi (sorghum-based) and fufu (cassava-based) were EPS producers (Adebayo-tayo and Onilude, 2008). *L. delbrueckii* ssp. *bulgaricus* NCFB 2772 was found to enhance to a greater extent the viscosity of an oat-based medium when glucose was present as a supplementary carbon source instead of fructose, which was seen to cause the release of EPS with lower relative molecular mass (Grobben et al., 1997). Similarly, Mårtensson et al. (2002) studied the possibility of developing a yogurt-like ropy product derived entirely from oats and water by employing an EPS-producing strain of *Pediococcus damnosus* in combination with an ordinary yogurt starter culture. A sensory preference test successfully showed no significant difference between the flavored, nondairy product and a dairy equivalent control. Similarly, the textural properties of a beverage formulated with gelatinized emmer flour (30% w/w in tap water) and added sucrose (10% w/w) were enhanced using EPS-forming species of *Weissella cibaria* as inoculum. The fourfold viscosity increase, compared to a control fermented by EPS-negative *L. plantarum* strain, conveyed a texture similar to yogurt to the final product (Coda et al., 2011). In a recent study, the potential of two cereal-associated *W. cibaria* strains to produce exopolysaccharides in situ during the development of a prebiotic drink based on barley malt extract was examined (Zannini et al., 2012). The higher viscosity positively influenced the mouthfeel of the beverage. Some cereal flours, when mixed with water, can lead to a porridge-like texture because of the high content of molecules like starch and β-glucans that have a viscosity-enhancing effect (Lorri and Svanberg, 2009). This characteristic is considered important in certain African countries, where maize, sorghum, or millet porridges represent a crucial energy source as weaning food for young children (Humblot et al., 2014). In order to maintain a high-energy density in these formulations without the need for watering down, LAB with enzymatic activity have been employed for partially degrading these biopolymers (Onyango et al., 2004). Among these, amylolytic LAB (ALAB) that are able to degrade polysaccharides have been isolated from many traditional beverages (Guyot, 2012). The biodiversity of ALAB is quite limited and the most prominent ones belong to the species *Lactobacillus manihotivorans*, *Lactobacillus fermentum*, *Lactobacillus amylovorus*, *Lactobacillus amylophilus*, *L. plantarum*, and *Lactobacillus amylolyticus* (Reddy et al., 2008).

Raw cereals carry very low levels of organoleptic-active compounds, and in this form, confer flat, and often unpleasant odors and flavors (Peyer et al., 2016). The bitterness and astringency carried by certain phenolic compounds found in the outer layers of whole grains can also lead to poor acceptance (Peyer et al., 2016). Together with other technological processes such as boiling, toasting, and roasting (Coda et al., 2011), fermentation has been used to improve sensorial and textural properties of cereal-based beverages (Nionelli et al., 2014). Investigations on sensorial changes in cereal matrices after LAB fermentation in cereal matrices have been initially done in relation to off-flavor formation during microbial spoilage in beer (Peyer et al., 2016). More recently, research has concentrated on the flavor and textural changes caused by the inoculation of selected LAB (Peyer et al., 2016), particularly in the case of probiotic addition (Coda et al., 2011; Salmerón et al., 2014), or for quality improvement of traditional cereal-based fermented beverages (Blandino et al., 2003) (Table 10.1). Overall, it was demonstrated that the use of defined starter cultures led to a product with better appearance, aroma, taste, and acceptability than spontaneously fermented beverages (Peyer et al., 2016). The majority of work done on novel cereal-based fermented beverages has used *L. plantarum* as starter culture because of its robustness under conditions of low pH (Charalampopoulos et al., 2002). This trait often gives this species a competitive advantage against other autochthonous microorganisms present on the grains, and the ability to deliver a pleasant organoleptic profile in the form of "dairy"-related flavors (e.g., diacetyl, acetoin, acetaldehyde) (Prado et al., 2008; Salmerón et al., 2015). However, a defined starter does not preclude the release of specific flavors when inoculated in different cereal matrices. The microbial flavor compounds released by *L. plantarum* NCIMB 8826 after fermentation of four different gruels (oat, wheat, barley, and barley malt) were present at varying concentrations depending on the cereal used (Salmerón et al., 2009). Moreover, none of the metabolites were common for all substrates, indicating a complex flavor-formation interdependency that exists between bacterial culture and substrate components (Peyer et al., 2016). Carbohydrates, amino acids, and other chemical compounds (e.g., organic acids, fatty acids) present in cereals, or released from LAB during fermentation, can be channeled into different metabolic pathways that ultimately lead to specific organoleptic compounds (Gänzle et al., 2007).

Organic acids (lactic, acetic) are the main compounds deriving from the sugar metabolism of LAB. The extent to which lactic and acetic acid accumulate depends primarily on the metabolism of the specific starter and on the substrate supply, but fermentation conditions, e.g., temperature, buffering capacity, affect culture viability (Helland et al., 2004) and determine the extent of acids released in the medium as well. For example, if compounds that can function as alternative electron acceptors are present in the medium, pyruvate can be channeled into alternative metabolic pathways (Liu, 2003), increasing the ratio of acetic to lactic acid released in the media (Kandler, 1983). Lactic acid, quantitatively the most important organic acid found after LAB fermentation, is odorless but in aqueous solution imparts a mild acidic note (Hartwig and McDaniel, 1995). The "sour" perception of lactic acid in beverages carries important thirst-quenching properties and consequently has been exploited in novel refreshing products (Warner, 2010). Acetic acid, compared to lactic acid, is released in lower concentrations, but because of its lower taste threshold and higher volatility, it can become perceptible as pungent-sour with a "cider-vinegar" aroma (Burdock, 2002). Finally, the importance of the pH after fermentation, often found between 3.0 and 4.5, as a factor influencing the final acceptance of a novel beverage should also be considered (Salmerón et al., 2015). In fact, the increase in sourness coincides with a general decrease in sweetness (McFeeters, 2004), unless the release of sugar moieties exceeds again the sugar consumption, leading to a consequent increase of the sweet taste (Mugula et al., 2003).

The contribution of LAB to flavor and taste on volatile and nonvolatile fractions of cereal-based beverages has been recently reviewed (Peyer et al., 2016). The nonvolatile fractions include primarily sugars and some carboxylic acids that contribute to the sweet and sour taste of the beverages. The volatile fraction, including compounds perceived as odor and flavor, comprises carboxylic acids, alcohols, aldehydes, ketones, and esters (Peyer et al., 2016). The main volatile compounds of cereal-based beverages fermented by LAB have been mostly associated with the carbohydrate and amino acid metabolisms (Peyer et al., 2016). Diacetyl (butane-2, 3-dione) is a ketone responsible for a butterscotch-like aroma (Burdock, 2002), mainly formed during sugar, citrate, and amino acid catabolism (Hugenholtz et al., 2000). Metabolically related to diacetyl are the less flavorsome acetoin, formed by the reduction of diacetyl or after enzymatic decarboxylation of α-acetolactate, and 2,3-butanediol, which results from the reduction of acetoin (Axelsson, 1998). Although considered as being off-flavors in beer (Bokulich and Bamforth, 2013), these low-molecular-weight compounds are also responsible for mellowing the flavor during cereal fermentation (Mugula et al., 2003). Only some LAB species, e.g., *Lactococcus lactis*, *L. plantarum*, and *Oenococcus oeni*, are able to metabolize citrate to pyruvate that can be redirected into the acetoin/diacetyl pathway (Hugenholtz, 1993). High levels of diacetyl and acetoin can also be found due to alternative metabolic patterns (Peyer et al., 2016). Acetaldehyde is a highly volatile aldehyde formed from pyruvate or threonine catabolism (Ardö, 2006), which has been described as delivering a pungent, fruity (green apple) flavor with sweet notes (Mahattanatawee et al., 2005). High level of acetaldehyde found after fermentation of a malt-based beverage with *L. plantarum* NCIMB 8826 positively contributed to the acceptance of the beverage (Salmerón et al., 2015). Amino acids play a central role as flavor-forming substrate in LAB (Gänzle et al., 2007). Besides possessing taste properties of their own (e.g., sweet, bitter, sulfurous, and umami) (Solms, 1969), amino acids serve as substrate for Maillard reactions that can accumulate organoleptic-active carbonyl compounds, heterocycles as well as melanoidins (Pozo-Bayón et al., 2006). The by-products of amino acid catabolism in LAB have been repeatedly reported as important flavor-active compounds in liquid cereal-based fermentations (Coda et al., 2011; Mugula et al., 2003; Muyanja et al., 2012). Aldehydes and alcohols can be released from the catabolism of the amino acids, after conversion into α-ketoacids by means of aminotransferases, and subsequent decarboxylation into aldehydes (Ardö, 2006). The reduction of these compounds into alcohols, however, was often assigned to endogenous yeasts present in the raw cereals (Muyanja et al., 2012). Microbial fermentation can lead to the activation of the endogenous proteinases (pH optimum between 4 and 5 in wheat, rye, and barley) (Belitz et al., 2009), but LAB can also actively increase the fermentable nitrogen level providing proteases and peptidases (Coda et al., 2012; Thiele et al., 2002). Finally, free fatty acids such as oleic and linoleic acid can act as precursors for methylketones, alcohols, and lactones (Smit et al., 2005). However, because of the generally low-lipolytic activities of LAB, these volatiles are formed by other microorganisms associated with food production, e.g., molds in cheese. Because of the relatively low concentration of lipids in cereals, volatiles derived from lipolysis metabolism have not been studied in detail during liquid cereal fermentations (Peyer et al., 2016).

10.3 YOGURT-LIKE BEVERAGES MADE WITH OAT

Among the cereal grains, oat is known to provide a vast range of health benefits, including reduced symptoms of diabetes and obesity (Tapola et al., 2005; Zdunczyk et al., 2006). Oat is one of the major sources of β-glucan, a cell wall polysaccharide recognized as the main functional component of cereal fiber and primarily responsible for these health benefits (Angelov et al., 2006). β-Glucan content in oat is about 2.3%–8.5% (Flander et al., 2007) and its structure consists of consecutively linked (1/4)-β-D-glucosyl residues in oligomeric segments that are separated by single (1/3)-linkages along the polymer chain backbone (Lazaridou et al., 2004). According to the definition of the American Association of Cereal Chemists, oat β-glucan can be considered as dietary fiber and therefore contribute to "promote beneficial physiological effects including laxation and/or blood cholesterol attenuation and/or blood glucose attenuation" (AACC, 2001; Rasane et al., 2015). The health effects of oat β-glucan consumption were officially recognized first by the US Food and Drug Administration (US FDA, 1997) and more recently by the European Food Safety Authority (EFSA, 2010) according to which a daily consumption of 3 g of β-glucan leads to the reduction of blood plasma cholesterol concentration, which is a major risk factor for the development of coronary heart disease (Lazaridou et al., 2014). Moreover, oat β-glucan has potential anticarcinogenic property, as it reduces compounds that are causative agents of colon cancer (Butt et al., 2008), reduces blood cholesterol levels (Ripsin et al., 1992; Amundsen et al., 2003), and decreases blood pressure (Maki et al., 2007).

Due to all these benefits, oat has been widely exploited for making beverages in recent years, particularly, as a fermented beverage with functional properties (Marsh et al., 2014; Corbo et al., 2014). Moreover, with its high content of dietary fibers, including β-glucan, inulin, and resistant starch, oat is a good source of fermentable carbohydrates for colonic bacteria, which can act also as prebiotics in association with probiotic microorganisms (Gibson et al., 1995; Charalampopoulos et al., 2002b). As previously shown, probiotic and prebiotic effects might be additive or even synergistic. Their combination could benefit the host by improving survival and implantation of microorganisms in the gastrointestinal flora, by selectively stimulating the growth or activity of health-promoting bacteria in the intestinal tract, and by improving the gastrointestinal tract's microbial balance (Roberfroid, 2000).

For all these reasons, probiotic fermented beverages are one of the most important categories of the functional food segments (Sirò et al., 2008). The attractiveness and high consumer acceptance of fermented functional beverages relies on the activity of specific living microorganisms, which is the main reason for their functionality (Gobbetti et al., 2010). Indeed, probiotic bacteria such as *Bifidobacterium* spp. and LAB have been used as starter cultures for the production of fermented oat beverages, delivering microorganisms to the gut and providing enhanced nutritional profile (O'Connor et al., 2005).

10.3.1 OAT-BASED PREBIOTIC, PROBIOTIC, AND SYMBIOTIC BEVERAGES

The nutritional properties of oat and the physiological characteristics of specific strains of LAB and bifidobacteria make oat probiotic yogurt-like beverages a suitable contributor to enhancing and maintaining health and improving the quality of life. Recently, several studies have shown the positive effect of oat fermentation by probiotic strains. For probiotic products, viability at a minimum of 6 log cfu/mL and activity of the bacteria are important parameters, as they must survive in the food during shelf life and during transit through the acidic conditions of the stomach in order to perform their beneficial effects (Tamime et al., 2005). Several probiotic species of different origin have been successfully grown in oat substrate, such as *Lactobacillus rhamnosus* are able to grow up to 7 log cfu/g and dominate in oat bran fermentation and *L. plantarum* in whole-grain fermentation, reaching viable cell counts of 10 log cfu/mL after 24 days of refrigerated storage (Loponen et al., 2007). In the latter case, the final result was a symbiotic product, combining the positive effects of probiotics with the oat prebiotic β-glucan, increasing the potential health benefits (Angelov et al., 2006). A symbiotic interaction between prebiotics, such as β-glucan, and probiotics might result in increased health benefits for the host, by promoting the growth of the bacteria and releasing low-molecular fatty acids (butyrate, acetate, and propionate). The release of short-chain fatty acids can provide energy and acidify the bowel thereby producing a less advantageous environment for pathogens, and at the same time exert potential anticarcinogenic effect (Salminen et al., 1998; Gallaher, 2000). Therefore, the presence of both β-glucan and probiotic during gastrointestinal digestion is a strategy to further enhance the health attributes of oat beverages. The fermentation of oat-based substrates with *Lactobacillus reuteri*, *L. acidophilus* and *Bifidobacterium bifidum* delivered a yogurt-like product with acceptable appearance and taste and small repercussion on the β-glucan content after storage (Mårtensson et al., 2001). Similarly, a probiotic strain of *L. plantarum* was used to produce a functional oat drink having β-glucan content of 0.31%–0.36%, which remained unchanged throughout fermentation and storage (Angelov et al., 2005).

Symbiotics can have effect in the reduction of osteoporosis and improvement of mineral absorption (Scholz-Ahrens et al., 2007). Oat-based symbiotic yogurt-type beverage containing different LAB or bifidobacteria have been further developed and improved in the last years (Table 10.1). An oat yogurt containing *L. plantarum*, *Lactobacillus paracasei* ssp. *casei*, and *L. acidophilus* was developed at the University of Vermont, USA, and was shown to have some epithelial adhesion to Caco-2 cell lines indicating potential for intestinal colonization (Gokavi et al., 2005). In another case, an oat symbiotic yogurt-like beverage (Oagurt) was developed using probiotics (*L. acidophilus*, *Lactobacillus casei*, and *Bifidobacterium*), fortified with inulin to increase soluble fiber, minerals, and vitamins, and to which prepolymerized whey protein was added to improve the textural properties (Walsh et al., 2010). More recently, a yogurt-like beverage made with oat flakes and fermented by *L. plantarum* showed an increase in the polyphenols' availability and antioxidant activity as well as reduced hydrolysis index in vitro, highlighting the high-nutritional potential of these beverages (Nionelli et al., 2014).

An example of the market relevance of oat functional beverage is represented by Proviva (Skane Dairy, Sweden) and Yosa (Bioferme, Finland), deriving from wholegrain fermented oat, similar to flavored yogurt or porridge, containing *L. acidophilus* and *B. bifidum*. Yosa content of oat fiber and probiotic combines the effect of β-glucan on cholesterol reduction and the effect of LAB benefits to maintain and improve the environment in the intestinal balance of the consumer (Blandino et al., 2003; Salovaara, 1996). The potential beneficial effects of functional oat beverages on human health encourage further research for its complete exploitation in clinical usages.

10.4 YOGURT-LIKE BEVERAGES MADE WITH OTHER CEREALS: FROM TRADITIONAL TO INNOVATIVE PRODUCTS

On a worldwide basis, maize, rice, and wheat are the prevailing crops in terms of area reserved for cereal cultivation and total cereal production (Poutanen, 2012). However, ancient and/or minor cereals, such as kamut, spelt, einkorn, millet, and sorghum, and pseudocereals, such as quinoa, amaranth, and buckwheat, have generated great interest, particularly in Western countries, because of their higher content in beneficial minor components (dietary fiber, resistant starch, minerals, vitamins, phenolic compounds) (Coda et al., 2014) compared to staple grains (wheat, maize, rice), and the possibility to fulfill specific dietary needs, such as low gluten or gluten free (Zannini et al., 2012).

Cereals are considered a suitable substrate for the production of probiotic products (Kandylis et al., 2016). In addition, their consumption has been associated with the reduction of the risk of several chronic diseases (Kandylis et al., 2016). For these reasons, besides several probiotic beverages that are being produced worldwide, nowadays the scientific community and the food industry try to produce innovative beverages based on single or multicereals (Kandylis et al., 2016) (Table 10.1).

In many traditional cereal-based beverages (Table 10.1), popular in tropical regions of Asia and Africa, the grains are often heated, mashed, and sometimes filtered (Marsh et al., 2014). Not all the traditional cereal-based beverages are characterized by a yogurt-like texture. For the inoculation, the backslopping procedure is quite common, but the microbial populations responsible for the fermentation of these beverages are not well characterized (Marsh et al., 2014). Boza, consumed in Bulgaria and Turkey, is generated through the fermentation of a variety of cereals including barley, oat, rye, millet, maize, wheat, or rice, with the specific composition affecting the viscosity, fermentability, and content of the final beverage (Akpinar-Bayizit et al., 2010). The cereal is boiled and filtered, a carbohydrate source is added, and the mixture can be left to ferment spontaneously or with the use of backslop. *S. cerevisiae*, *Leuconostoc mesenteroides*, and *Lactobacillus confusus* were often isolated from Boza (Zorba et al., 2003). Togwa, a sweet and sour, nonalcoholic beverage, is one of the better studied African cereal beverages. This is produced from the flour of maize, sorghum, and finger millet and, sometimes, cassava root. The chosen substrates are boiled, cooled, and fermented for approximately 12 h to form a porridge, which is then diluted to drink (Kitabatake et al., 2003). Mahewu is similar in that maize or sorghum meal is fermented with millet or sorghum malt, and is available commercially (Mugochi et al., 2001). Bushera is generally prepared from germinated or nongerminated sorghum grains, and fermented for 1–6 days (Muyanja et al., 2003). These beverages are often used to wean children and also as a high-energy diet supplement. Koko sour water is the fermented liquid water created in the production of the fermented porridge koko. This contains a high LAB density and is used by locals to treat stomachaches and as a refreshing beverage (Lei and Jakobsen, 2004). Kvass is a fermented rye bread beverage common in Russia, which has seen much commercial success. The beverage can have a sparkling, sweet or sour, rye bread flavor (Jargin, 2009). Amazake is a sweet fermented rice beverage that is the nonalcoholic precursor to sake, produced in Japan. Steamed rice is mixed with rice-koji (*Aspergillus* mycelia and rice) and water, and is heated to 55–60°C for 15–18 h. Enzymes break down the rice and form glucose content of approximately 20%. Amazake is highly nutritious and is consumed for its purported health benefits (Yamamoto et al., 2011). Pozol, common in southeastern Mexico, has quite a peculiar method of production, in which maize grains are heat-treated in an acid solution, ground, and shaped into dough balls. These are then wrapped in banana leaves and fermented for 2–7 days, successively diluted with water and consumed as a beverage. Pozol hosts a variety of microorganisms including LAB, non-LAB, yeasts, and other fungi (Ben Omar and Ampe, 2000). Rice is a common substrate for the production of beverages especially in Asia and South America. Haria is a rice-based ethnic fermented beverage of East-Central India. It has low alcohol content of 2–3% (v/v) and titratable acidity of 1.42% (Ghosh et al., 2014). During fermentation yeast, mold, LAB, and *Bifidobacterium* spp. are present and through synergistic actions define the final characteristics of haria. They convert starchy materials of rice to malto-sugars and enrich the final product with antioxidants and bioactive substances (Ghosh et al., 2014). Similar characteristics were observed in another rice-based beverage of North Western Himalayas in India, the chhang (Thakur et al., 2015). A rice-based beverage with the name chicha was also produced in Brazil by Umutina Brazilian Amerindians (Puerari et al., 2015). LAB and *Bacillus* spp. were the dominant microorganisms in the beverage. The final product has no ethanol but glycerol and low acidity and is characterized as an acidic nonalcoholic beverage (Kandylis et al., 2016).

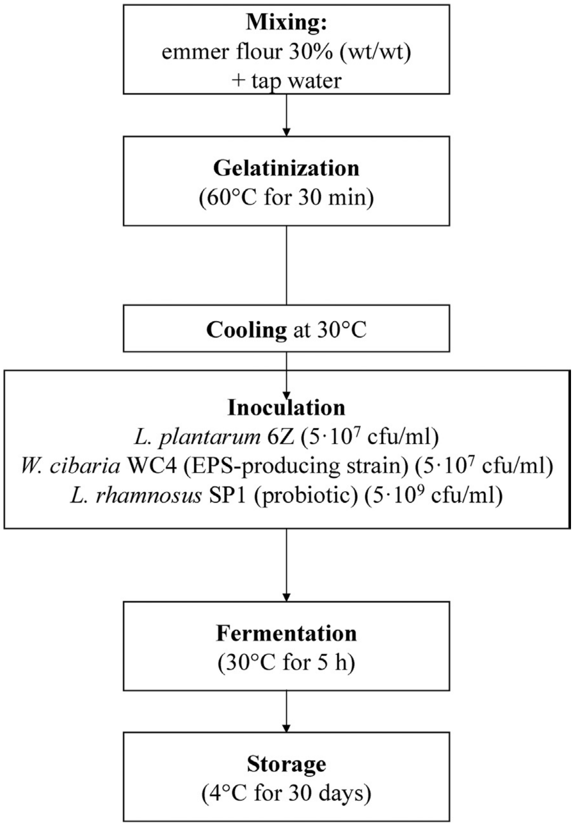

FIGURE 10.1

Protocol for making a yogurt-like beverage using emmer (*Triticum dicoccum*) flour, as proposed by Coda et al. (2011). A pool of LAB strains, selected on the basis of protechnological, probiotic, and EPS-production properties, was used as starter for fermentation.

A yogurt-like beverage was produced using emmer (*Triticum dicoccum*) flour and a *L. plantarum* strain isolated from the same matrix and selected on the basis of different protechnological properties (Coda et al., 2011) (Fig. 10.1). The substrate, including 30% of emmer flour in water, was gelatinized prior to inoculation (Coda et al., 2011). The beverage texture was improved using an EPS-producer LAB strain, and it was found that the matrix allowed a proper survival of a probiotic *L. rhamnosus* during long-time storage at 4°C. Several nutritional properties, including a low-hydrolysis starch index, high-phytase activity, and high concentration of essential amino acids characterized the novel beverage (Coda et al., 2011). A beverage produced with malt and *L. acidophilus* showed high acceptance from the consumers, also due to high-acetaldehyde concentrations and mild pH values (Salmerón et al., 2014, 2015). Recently, cereal (rice, barley, emmer, and oat) and soy flour and grape must were used for making vegetable yogurt-like beverages (Coda et al., 2012). Two selected strains of *L. plantarum* were used for lactic acid fermentation, according to a process that included the flour gelatinization. All the yogurt-like beverages had values of pH lower than 4.0 and both the starters remained viable at c. 8.4 log cfu/g throughout the storage. During fermentation, LAB utilized glucose, fructose, and malic acid, which were supplied through grape must (Coda et al., 2012). Compared to control beverages made without bacterial inoculum, an increase of total free amino acids was found during fermentation and storage. Also the concentration of polyphenolic compounds and ascorbic acid was higher when the LAB were used as starters, consequently leading to high antioxidant activity (Coda et al., 2012). Among the different cereal substrates, the beverages made with the mixture of rice and barley or emmer flours appear to possess the best combination of textural, nutritional, and sensory properties (Coda et al., 2012).

From the technological perspective, the influence of containers, substrates, metabolites, and fermentation kinetics, together with the peculiar microbiota of the traditional beverages, are the key factors to define the role of the different microorganisms in fermentation processes, and their contribution to the final features of the products (Marsh et al., 2014). At the same time, the characterization of the nutritional and functional properties is fundamental for commercialization and is required by the consumers. Nevertheless, the trend of the scientific community and the food industry is to use traditional beverages as a model for the development of new products, since the evaluation of the functional and sensory properties in controlled fermentations is easier (Marsh et al., 2014).

10.5 FUTURE TRENDS

Cereals are a very good alternative to dairy and can have multiple beneficial effects, which make them very suitable for functional beverages. Fermented cereal beverages have high-mineral content, and generally a lower-fat content than their dairy-based counterparts. They also naturally provide plant functional components, such as fiber, vitamins, minerals, flavonoids, and phenolic compounds, which can affect oxidative stress, inflammation, hyperglycemia, and carcinogenesis (Wang et al., 2014). The development of functional fermented-cereal products through application of defined LAB meets the current demand for healthier and diversified foods (Peyer et al., 2016) although the research in the area of nondairy probiotic beverages is currently in an early stage (Kandylis et al., 2016). The selection of specific microbial starters will be crucial to deliver specific healthy properties but also to reproduce the desirable characteristics of traditional health-promoting beverages in industrial scale (Marsh et al., 2014). The full prediction of the final sensory attributes, for example, is still a very complex task due to dynamic interactions between starter cultures, substrate, and fermentation conditions. The use of a broader array of cereal substrates inoculated with single or combined starters could be investigated under different conditions (Peyer et al., 2016), since the process conditions can be modulated to obtain a large range of specific effects on the sensory, technological, nutritional, and functional aspects of the final product.

Therefore, different aspects must be taken into account for the design of novel cereal-based yogurt-like beverages, at scientific and industrial levels, such as the characterization and the standardization of the bioactive compounds, the selection of starters able to produce bioactive compounds and to positively affect the organoleptic and texture features, the survival of probiotic organisms during storage, the application of natural biopreservatives to improve the natural image of the functional beverages, the bioavailability and metabolism of functional ingredients, the safety aspects related to the consumption, and the formulation of value-added products based on traditional fermented beverages (Corbo et al., 2014).

Nevertheless, the future of functional yogurt-like beverages, including their commercial success, mainly depends on the unequivocal demonstration of their efficacy in promoting health (Corbo et al., 2014). Thus, the cooperation between the food industry and the scientific community is strictly necessary to provide scientific evidence of the health claims, to improve the biotechnological tools for their large-scale production and distribution, and to enhance their attractiveness and sensory properties.

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