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Potential use of lactic acid bacteria for reduction of allergenicity and for longer conservation of fermented foods

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The interest of consumers for diverse fermented foods has increased in recent years thanks to the positive perception of their impact on consumer health considered as beneficial. Hence, there is an evident need for search of novel ways and for new food preservation agents of natural origins. In this aspect, lactic acid bacteria are very good candidates. It should be highlighted also that the onset of food allergies is rising significantly in recent years. The reduction of the immunoreactivity of food proteins could be achieved thanks to preprandial proteolysis occurring in fermented dairy (and other food) systems changing the allergen presentation or cleaving the allergenic protein epitopes, and produce hypoallergenic products.

Introduction

Lactic acid bacteria (LAB) are found in a plethora of niches, including plant material, fermented dairy, vegetable and meat products, and sourdough breads. Foods fermented by LAB are rendered more adapted for longer preservation and have improved textures, flavors and tastes. Hence, a variety of LAB, notably *Lactococcus* and *Lactobacillus* spp., are used as starter cultures for the production of fermented foods and they persist in them in high numbers.

The production of fermented foods is one of oldest food processing technologies known to man. However, according to Ayurvedic texts the use of fermented foods is subject to many conditions mainly linked to the type of the season of the year and human individual humoral constituency "dacha".

Important agricultural and economic crises in the recent years, such as mad cow, foot and mouth disease, porcine and avian influenza, etc., indicate how crucial is the sanitary status of the consumed food products and how deep and economically devastating may become outbreaks of food borne infections and resulting consumer fears and panics. A variety of LAB strains were used in different countries and in different products for fermentation such as *Lactococcus lactis*, *Streptococcus thermophilus*,

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Lactobacillus bulgaricus, *Lactobacillus sakei*, *Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, etc. (Knorr, 1998; Caplice & Fitzgerald, 1999).

Probiotics have now been defined as live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host (Reid *et al*., 2003). Today, fermented dairy foods supplemented with probiotics have grown into a multi-million Euro business (Buss, 2004). In most cases, these functional foods include LAB and bifidobacteria that are marketed as probiotics. A probiotic can beneficially affect the health of the host when it is ingested at certain levels by preventing the growth of harmful bacteria thanks to competitive exclusion and thanks to the generation of organic acids and antimicrobial compounds (Salminen *et al*., 1996). The desirable probiotics should have the following properties: resistance to acid and to bile toxicity, adherence to human intestine cells, colonization in human guts, antagonism against pathogenic bacteria, production of antimicrobial substances, and immune modulation properties (Brassart & Schiffrin, 2000).

It should be highlighted also that the onset of food allergies is rising significantly amongst the world populations. It is concerning particularly infant group of the European Community population. In some cases, very severe allergic reactions against consumed foods and to dairy proteins in particular, lead to anaphylaxis. In this context well described and well studied influence of the fermentative processing of foods, and in particular the dairy food systems, could help to circumvent or limit the severity of some adverse allergenic reactions. This could be achieved thanks to pre-prandial proteolysis occurring in fermented dairy (and other food) systems changing the allergen presentation or cleaving the allergenic protein epitopes. Hence, it could be expected that well managed fermentative transformations of dairy (and other food) products with appropriate LAB strains, could produce hypoallergenic products at least for some classes of allergic patients.

Fermented foods

The word fermentation has had many meanings in the past. In the broad sense, in which the term is commonly used, it is "a process in which chemical changes are brought about in an organic substrate through the action of enzymes elaborated by micro-organisms." Fermentation not only increased shelf life and microbiological safety of foods but also makes some foods more digestible (Caplice & Fitzgerald, 1999). Foods fermented by LAB are largely consumed. LAB are heterogeneous group of bacteria used as starter culture for different foods such as dairy, meat, vegetables and cereals for fermentations (Yamamoto, Togawa, Shimosaka, & Okazaki, 2003; Caplice & Fitzgerald, 1999). They also contribute to various industrial applications in food and beverage fermentation, bulk and fine chemicals productions as well as in manufacture of pharmaceuticals (Zhu, Zhang, & Li, 2009). LAB are well known as producers of proteolytic enzymes, exopolysaccharides (Welman & Maddox, 2003) and many secondary metabolites such as antimicrobial compounds (de Vuyst & Leroy, 2007).

The specific antimicrobial properties of LAB used in the biopreservation of foods are to produce organic acids such as lactic, acetic and propionic acids, hydrogen peroxide, carbon dioxide (formed from heterolactic fermentation), diacetyl (product from citrate metabolism), reuterin (produced during stationary phase by anaerobic growth of *Lactobacillus reuteri* on the mixture of glucose and glycerol or glyceraldehydes) and various bacteriocins (de Vuyst & Vandamme, 1994).

Dairy products

LAB play an important role in the biochemical events taking place during cheese ripening and are widely used in the dairy industry for their milk acidification, flavor development, proteolysis and sometimes also for the protection against phage attack despite being also sensible to phagolysis (Caplice & Fitzgerald, 1999; di Cagno *et al*., 2003). Different *Enterococcus* spp. strains were found to have a long history of safe use in food (Sarantinopoulos *et al*., 2001; Hugas, Garriga, & Aymerich, 2003). The positive action of enterococci on cheese taste and flavor originates from specific biochemical activities such as lipolysis, citrate utilization, production of aromatic volatile compounds, proteolysis and antimicrobial antagonism (Foulquié Moreno, Sarantinopoulos, Tsakalidou, & de Vuyst, 2006). Bacillar Lactic Acid Bacteria are also contributing intensively to the formation of sensory qualities of dairy products for example by proteolysis (El-Ghaish *et al*., 2010b) albeit their antimicrobial activities are somewhat less powerful than those found in cocci.

Proteolysis is considered to be one of the most important biochemical processes involved in the manufacture of many fermented dairy products, irrespective of the contribution of the proteolytic/peptidolytic enzymes of LAB to organoleptic properties of the final milk products. The ability to produce extracellular proteinases is a very important feature of LAB. They catalyze milk proteins, providing the amino acids essential for growth of LAB (Fira *et al*., 2001). Proteolysis by microbial enzymes in yogurt is a desirable process improving milk digestibility and enhancing nutritional quality of yogurt. It is accepted that proteolytic system of LAB degrades proteins and hence, changes the texture, the taste and the aromas of fermented products.

Allergy to cow milk (CMA) concerns ~2.5% of children below 3 years of age, hence the feeding of an infant allergic to cow milk casein may create serious trouble. Most studies revealed that caseins and β-lactoglobulin (β-lg) are the main allergens in cow milk (Cocco, Järvinen, Sampson, & Beyer, 2003; Gaudin *et al*., 2008). Different attempts have been made to reduce the allergenicity of dairy proteins, and various technological processes have been applied. Attempts to modify the protein components of cow milk in an effort to reduce their allergenic potential have included the application of heat treatment, enzymatic

treatment with a variety of enzymes and some combination of these processes, such as heating and glycation (Taheri-Kafrani *et al*., 2009). Heating has only a small effect on the antigenic/allergenic potential of cow's milk proteins, even though some authors have shown a reduction in whey protein antigenicity. Furthermore, heating processes can only modify conformational epitopes, which lose their binding capacity to specific IgEs. However, linear epitopes remain unaffected by structural changes and maintain their allergenicities after heating. Milk proteins contain both types of epitopes and, even though a slight reduction of antigenicity can be observed in case of whey proteins, only very insignificant changes in binding properties were reported for caseins (Restani, Ballabio, di Lorenzo, Tripodi, & Fiocchi, 2009).

Another method for reduction of antigenic/allergenic properties of milk proteins is bacterial fermentation. During microbial fermentations proteolytic enzymes can be produced and they can degrade milk protein allergens. Proteolytic systems of lactobacilli are complex and are composed of proteinases and peptidases with different sub-cellular locations. Cellular proteases grown on milk showed similar hydrolytic activities toward α_S- and β-caseins. Proteolysis is often followed by a reduction of the number of epitopes and consequently by a decrease in allergenicity of hydrolyzed proteins or could contribute to preventing allergenic problems frequent in children under 3 years of age due to poor digestion of milk proteins. Protein hydrolyzates are also included in specific formulations, as well as hypoallergenic infant formulas to reduce their antigenicity as compared with intact protein (Bertrand-Harb, Ivanova, Dalgalarrondo, & Haertlé, 2003; Cocco *et al*., 2003; Peñas, Préstamo, Baeza, Martínez-Molero, & Gomez, 2006; Pescuma, Hébert, Mozzi, & Font de Valdez, 2010; Pescuma *et al*., 2009, 2011; El-Ghaish *et al*., 2010a, 2010b, 2011b).

It was already reported that some LAB reduce the antigenic response of milk proteins (Wróblewska, Jedrychowski, & Bielecka, 1995). The reduction of milk protein antigenicity depends on the species of LAB and on conditions of fermentation (Kleber, Weyrich, & Hinrichs, 2006; Bu, Luo, Zhang, & Chen, 2010).

Lactic acid fermentation was applied to reduce the β-lg antigenicity of sweet whey and skim milk, and furthermore, its products were reported to have beneficial effects on the consumer including the activation of the immune system (Shida *et al*., 1998). The whey protein β-lg is in about 80% of all cases the main cause of milk allergies in children and infants. β-Lg is the major whey protein in milk and in dairy products and it is of particular interest since it is the only whey protein of cow's milk absent in human milk. Kleber *et al*. (2006) studied the ability of some LAB strains to reduce the antigenicity of β-lg in skim milk and in sweet whey by indirect competitive ELISA, using polyclonal antibodies. They observed the reduction of antigenicity of β-lg exceeding 70% in sweet whey and higher than 90% in skim milk compared to the initial value. In addition, the synergic reduction of the antigenicity was observed when co-cultures of LAB were used. The proteases produced by different LAB are more or less specific and the using of co-cultures yields higher hydrolysis of native allergens as well as of their epitopes. Fermentation of sterilized cow's milk using a mixture of meso- and thermophilic LAB resulted in almost complete disappearance of antigenicity of alpha-lactalbumin (α-lac) and β-lg (ELISA, rabbit antibodies) (Jedrychowski & Wróblewska, 1999). Different studies revealed that the combination of *Lactobacilli* strains with *S. thermophilus* during fermentation of milk and whey resulted in higher immunoreactivity reduction of native allergenic proteins. It was found in a recent study (Bu *et al*., 2010) that combined strains of *Lactobacillus helveticus* and *S. thermophilus* were the most effective in the reduction of the antigenicity of α-lac and β-lg in skim milk as compared with *L. helveticus* and *S. thermophilus* alone. α-Lac antigenicity was reduced by 87% with combined strains fermentation by *L. helveticus* and *S. thermophilus*. However, α-lac antigenicity was inhibited by 71% and 49% with *L. helveticus* and *S. thermophilus* alone, respectively.

During fermentation, release of proteases and peptidases results in more effective hydrolysis of milk proteins with higher possibility for cleavage of epitopes. Nevertheless, it is also possible that by further cleavage of the peptides into smaller peptides and amino acids by peptidases, some hidden epitopes or linear epitopes could be exposed. Bu *et al*. (2010) found that at the beginning of the fermentation, antigenicity of β-lg decreased gradually but at longer fermentation time it slightly increased, what indicates that there was no linear correlation between the degree of hydrolysis of milk protein and the antigenicity of whey proteins during the fermentation (Bu *et al*., 2010). Ehn, Allmere, Telemo, Bengtsson, and Ekstrand (2005) used different strains of *L. helveticus* to hydrolyze milk whey proteins and observed 80% hydrolysis of β-lg. However, this did not affect the epitope recognition by IgE as judged by the unchanged inhibition pattern (ELISA, human serum) after this treatment. This suggests that the extracellular proteolytic activity in the fermentation process did not degrade enough the IgE epitopes. It is also possible that the degradation was only partial, leaving peptides long enough to bind the antibodies, or it is possible that better access to some buried internal epitopes compensated any hydrolytic reduction of external epitopes. It is also possible that the preheating temperature might have a significant impact on the changes of the antigenicity of whey proteins. Kleber *et al*. (2006) studied the effect of different LAB on the antigenicity of β-lg in skim milk heated 40 min at 90 °C. They found that the antigenicity of β-lg decreased by 84–98% as compared with unfermented skim milk. Jedrychowski and Wróblewska (1999) claimed that after the fermentation with LAB the antigenicity of sterilized milk (110 °C, 10 min) was reduced by over 99%. However,

when prick test with whey samples from fermented whole milk were done on allergic to milk patients their allergic reactions were only slightly attenuated. Further *in vitro* and *in vivo* studies are necessary to understand better the mechanism of allergenicity of dairy proteins and the methods to decrease it. Additionally, it would be interesting to study the combination of various methods, such as fermentation and hydrolysis with animal and plant proteases, heat treatment, high pressure, and microwave combined with enzymatic proteolysis.

In recent years, various bacteriocins produced by *L. plantarum* species, *Lactobacillus casei*, *Lactobacillus lactis*, *Leuconostoc lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, Lactobacillus delbrueckii subsp. *lactis* and *S. thermophilus* were isolated from fermented dairy products. These bacteriocins showed broad inhibition spectra including some pathogenic organisms in foods such as *Listeria monocytogenes*, *Escherichia coli*, Shigella spp., *Staphylococcus aureus* and *Escherichia aerogenes* (Luo *et al*., 2011; Xie *et al*., 2011). Some Enterococci also of dairy origin (*Enterococcus faecalis* and *Enterococcus faecium*) have also been reported to produce different types of bacteriocins (enterocins), with antimicrobial activity limiting food spoilage or inhibiting pathogenic bacteria such as *L. monocytogenes*, *Staphylococcus aureus* and Bacillus spp. (Batdorj *et al*., 2006; Giraffa, 2003; El-Ghaish *et al*., 2011a). A lot of research has been carried out on characterization of enterocins, or of enterocins-producing strains for potential applications in dairy technology. Several studies have demonstrated the inhibitory effect of enterocin-producing *Enterococcus faecium* or *Enterococcus faecalis* strains against dairy systems artificially contaminated by *Listeria monocytogenes* such as milk, soft cheeses and soy milk. The presence and anti-Listeria activity of enterocins produced by protective cultures in cheese was observed at the end of ripening while no or minor influences were observed on the organoleptic properties of the products. The potential technological applications of enterocins produced during cheese manufacture incline to propose enterococci as potential adjusted starter or protective cultures in cheese industry. Enterococci can propagate through intestinal or environmental contamination forming persistent colonies in raw foods and in processed food systems thanks to their ability to survive in adverse environmental conditions such as extreme pH, temperature and salinity. In Denmark, a fermented milk product containing *E. faecium* SF68 has been sold for several years because of its hypocholesterolemic effect on individuals (Oumer *et al*., 2001; Giraffa, 2003; Foulquié Moreno *et al*., 2006).

However, the use of *enterococci* as probiotics remains a controversial issue. While the probiotic benefits of some strains are well established, the emergence and the increased association of *enterococci* with human gastrointestinal diseases and multiple antibiotic resistances often present in them raised concerns of their use as probiotics. The fear that antimicrobial resistance genes or genes encoding virulence factors can be transferred to other bacteria in the gastrointestinal tract contributes to this controversy (Franz, Stiles, Schleifer, & Holzapfel, 2003).

Some LAB starters used in yogurt production (*S. thermophilus* and *L. bulgaricus*) give aroma and flavor (acetaldehyde) to final products. The starters of kefirs consist of characteristic 'Kefir grains', which are different from yogurt starters, contain the acid producing *L. lactis* and *Lactobacillus delbrueckii* subsp. *bulgaricus,* which also produce a small amount of ethanol. Koumiss, which is produced in Russia and Mongolia, is similar to kefir but uses mare's milk and the starter is composed of *Lactobacillus acidophilus* and *L. delbrueckii* subsp. *bulgaricus* plus several yeasts. The popularity of fermented milk drinks is increasing, not only because of their attractive taste but also because of the many health benefits reportedly associated with them (Caplice & Fitzgerald, 1999).

Meat products

The European Union countries are the major producers of fermented meat products, which account for 20–40% of the total processed meat (Hamm, Haller, & Gänzle, 2008). Starter cultures (LAB) play an important role during meat fermentation mainly in sausages for improving hygienic, standardized and sensory quality of the end product (Jiménez-Colmenero, Carballo, & Cofrades, 2001; Toldra, 2007). The metabolites of such LAB fermentation prevent the development of spoilage and pathogenic microflora by acidification of the product, also contributing to its color stabilization and texture improvement. Fermentation of meat causes number of physical, biochemical and microbial changes, which influence functional properties of the products. Meat protein degradation occurring during sausage fermentation may be ascribed to the synergistic action of meat endogenous proteases, LAB proteolytic activity and acid-induced changes arising from the bacterial fermentative metabolism. Also, the introduction of starter strains that possess amine oxidase activity might be a way of bigger decrease of the amount of biogenic amines produced *in situ* (Martuscelli, Crudele, Gardini, & Suzzi, 2000).

It is now clear that allergy to beef meat is relatively frequent displaying incidences between 3.3% and 6.5% among children with atopic dermatitis. Its incidence may reach 0.3% in the general population. The major beef allergen is bovine serum albumin. Beef-sensitive children are also reactive to ovine serum albumin, as well as to other serum albumins. Therefore, the use of alternative meats in beef-allergic children must be carefully evaluated on an individual basis. Because industrial heat processing and enzymatic digestion is more efficient than domestic cooking in reducing reactivity in beef-sensitive children, freeze-drying and homogenization may facilitate the introduction of processed beef into the diets of beef-allergic children (Fiocchi, Restani, & Riva, 2000).

Some LAB have also been found to produce bacteriocins being used as bioprotective culture to preserve fresh and

processed meat and fish (Castellano, Belfiore, Fadda, & Vignolo, 2008). LAB producing bacteriocins have been demonstrated to reduce the count of food borne pathogens such as *L. monocytogenes* by one log early in meat fermentation and this application of bacteriocinogenic cultures inhibits the development of spoilage and pathogenic micro-organisms (Ennahar, Sonomoto, & Ishizaki, 1999). Most of European fermented sausages formulated with nitrite are produced with added starter culture such as *Lactobacillus* and *Pediococcus* spp. (Caplice & Fitzgerald, 1999). The predominant species during lactic fermentation of sausages are psychrotrophic *Lactobacillus sakei* and *L. curvatus* (Hugas, Garriga, Aymerich, & Montfort, 1993). The application of bacteriocins in food preservation satisfies industrial and consumer's demands for extension of shelf-life of foods. However, the effectiveness of bacteriocins in food systems should be analyzed with respect of their environment, bacteriocin stability and/or the intensity of their synthesis (Fadda, López, & Vignolo, 2010).

Several bacteriocins may be very useful in food when used in the proper conditions. The use of pediocin PA-1 for food biopreservation has been commercially exploited and is covered by several U.S. and European patents (Ennahar, Sashihara, Sonomoto, & Ishizaki, 2000; Rodríguez, Martínez, & Kok, 2002). Lauková, Czikková, Laczková, and Turek (1999) examined the effectiveness of enterocin CCM 4231 in controlling *L. monocytogenes* contamination in dry fermented Hornád salami. Addition of enterocin reduced the counts of *L. monocytogenes* immediately after addition of the bacteriocin and after 1 week of ripening of the salami, the *L. monocytogenes* count in the control (without enterocin added) was significantly higher. Results of these studies were highly promising and they underline the fact that bacteriocinogenic strains of LAB may play positive role in the food industry as starter cultures, co-cultures, or bioprotective cultures improving food quality and safety.

Seafood products

The role of LAB in marine products is complex, depending on the fish species, treatment and storage conditions, bacterial species and strains, and interaction between the bacteria. The acidification process due to the lactic acid production as the major end-metabolite of the carbohydrate fermentation is one of the most desirable side effects of their growth, inhibiting micro-organisms including the most common human pathogens. The bioprotective potential of endogenous LAB in relation to pathogens and spoiling bacteria has often been highlighted. However, the technology is still in its infancy compared with foods dairy (carbohydrate content) and meat products (sugar and salt added). Although not usual, some applications of LAB in fermentation of marine by-products are described (Leroi, 2010). *L. plantarum* has been found in Atlantic salmon, Pollock, Arctic char and cod. Other authors have also reported the presence of *L. mesenteroides*, *Lactococcus piscium*, *Vagococcus salmoninarum*, *Lactobacillus fuchuensis*, *Streptococcus* spp., and *Weissella* spp. (Matamoros, Pilet, Gigout, Prévost, & Leroi, 2009b). Plantaricin has been used for the conservation of smoked salmon (Laursen *et al*., 2005; Matamoros *et al*., 2009a).

Vegetable products

Lactic fermentation of fruits and vegetables is the basis of traditional methods used for centuries to protect materials against deterioration. Today it is used as a method of choice for the production of food both tasty and well preserved (Buckenhüskes, 2001; Nout & Rombouts, 2000). Almost all vegetables can be fermented in brine. LAB play an essential role and convert sugars and other nutrients mainly in lactic acid. Fermented products of high economic importance are olives (Lavermicocca *et al*., 2002), cabbage (Buckenhüskes, 1997) and cucumbers (Atul & Ramesh, 2008). Microbial population of fresh material is dominated by aerobic bacteria and yeasts, while LAB are a minority. Later, in acid conditions when the sugar content is high, and low oxygen, the plant substrates undergo a spontaneous lactic fermentation. The high level of salt develops a high osmotic pressure promoting thus the development of LAB. In addition to the production of lactic acid, LAB also have the ability to produce hydrogen peroxide by the oxidation of NADH-reduced flavin. Also, some strains producing bacteriocins such as plantaricin contribute to the conservation of fermented products by inactivating spoilage bacteria. Carbon dioxide produced by hetero fermentative lactobacilli adds a supplementary preservative effect. The micro flora during fermentations is composed of lactic lactobacilli species such as *L. plantarum*, *Lactobacillus paracasei*, *Lactobacillus fermentum* and *L. brevis*, and of the genera of *Leuconostoc*, *Pediococcus* and *Weissella* (Buckenhüskes, 1997; Yang, Crowley, Borneman, & Keen, 2001).

Cereal products

Cereal fermentation is one of the oldest biotechnological processes, where both beer and bread were produced by combination of yeast and LAB fermentations (Poutanen, Flander, & Katina, 2009). LAB used in baking (Clarke & Arendt, 2005), play a role in modifying bread texture (Arendt, Ryan, & Dal Bello, 2007) and flavor (Ur-Rehman, Paterson, & Piggott, 2006). LAB produce lactic and acetic acids, decreasing the pH. Yeasts produce carbon dioxide and ethanol. The interactions between yeasts and lactobacilli are important for the metabolic activity of the sourdough. The changing conditions during fermentation contribute to the activation of enzymes present, and adjustment of pH to the performance of certain enzymes, such as amylases, proteases, hemi-cellulases and phytases (Poutanen *et al*., 2009). During rye sourdough fermentation, endogenous rye proteases, especially aspartic proteases, hydrolyze rye proteins, especially secalins and produce amino acids and small peptides, which act as flavor precursors (Tuukkanen, Loponen, Mikola, Sontag-Strohm, &

Salovaara, 2005). Celiac disease is a digestive disease that damages the small intestine and interferes with absorption of nutrients from food. People who have celiac disease cannot tolerate gluten, a protein present in wheat, rye, and barley. Gluten is found mainly in foods but may also be found in everyday products such as drugs, vitamins, and lip balms. The use of sourdough in baking of gluten-free bread has been efficient in improving product texture and to delay staling of gluten-free breads (Moore, Juga, Schober, & Arendt, 2007). Two studies (di Cagno *et al*., 2004; de Angelis *et al*., 2006) showed that pools of LAB (sourdough lactobacilli and commercial probiotic preparation) under specific processing conditions (long-time and semi-liquid fermentation) had the capacity to hydrolyze the wheat gliadin fraction improving their digestibility. The degradation of the cereal proteins in wheat and rye sourdough fermentations is a phenomenon, which strongly affects the flavor and texture of bread. Acidification and reduction of disulfide bonds of gluten by lactobacilli increase the activity of cereal proteases, which improves the digestibility of these proteins by the consumers. This may be used for production of new products (gluten intolerance) (Gänzle, Loponen, & Gobbetti, 2008). In the end, the proteolysis by LAB has been suggested as a new tool for preparation of acceptable food for fraction of consumers suffering from celiac disease. The use of selected sourdough cultures to eliminate risks of contamination by gluten and enhance the nutritional properties of gluten-free bread was highlighted by di Cagno *et al*. (2008). Also, cereal LAB fermentation may promote gut health in future nutritional applications (Poutanen *et al*., 2009).

Sourdough-associated LAB produce many antimicrobial substances, such as organic acids, CO_2, ethanol, hydrogen peroxide, diacetyl, fatty acids, phenyllactic acid, reuterin and fungicins (Schnürer & Magnusson, 2005). Among the organic acids, acetic and propionic acids produced by hetero fermentative LAB are more effective than lactic acid (Schnürer & Magnusson, 2005). Caproic acid produced by *Lactobacillus sanfranciscensis* CB1, together with a mixture of acetic, formic, propionic, butyric, and n-valeric acids, play a key role in inhibiting *Fusarium*, *Penicillium*, *Aspergillus* and *Monilia* growth in bread (Corsetti, Gobbetti, Rossi, & Damiani, 1998).

Conclusions

It is evident that not only organic acids, hydrogen peroxide, ethanol and diacetyl, but also additional LAB metabolites contribute to the antimicrobial capacities of starter cultures and to the final health status of produced foods. One group of such metabolites is composed by the bacteriocins. Because LAB strains are ‘generally recognized as safe’ (GRAS) in food production use, the detection and identification of bacteriocins produced by such bacteria have therefore received much attention, as these substances can be applied as ‘natural’ food preservatives.

During the fermentation by LAB the hydrolysis of milk proteins may have important effects on milk digestibility and milk proteins allergenicity. The use of proteolytic LAB in the dairy systems contributes to the decrease of allergenicity of the fermented milk products.

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