Lactic acid bacteria in sourdough fermentation; a safe approach for food preservation

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ABSTRACT

Bacterial starters have been developed for various fermented cereal products to improve their sensory and technological qualities. The earliest production of fermented foods was based on spontaneous fermentation due to the development of the microflora naturally present in the raw material. The quality of the end product was dependent on the microbial load and spectrum of the raw material. The direct addition of selected starter cultures to raw materials has been a breakthrough in the processing of fermented foods, resulting in a high degree of control over the fermentation process and standardization of the end product. The latter can contribute to the microbial safety or offer one or more organoleptic, technological, nutritional, or health advantages. Examples are lactic acid bacteria that produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, vitamins, enzymes, or that have probiotic properties.

Key words: Fermentation, sourdough, bread, lactic acid bacteria

INTRODUCTION

Food fermentation has been used for centuries as a method to preserve perishable food products. Fermentation was invented long before microorganisms were discovered, and therefore the process seemed mysterious. The need for an inoculum was understood and usually satisfied by keeping a sample from the previous production. This procedure is still in use for propagation of sourdough. With the discovery of microorganisms, it became possible to improve the products and the fermentation processes by using isolated and well characterized cultures. Lactic acid bacteria are widely used in the production of fermented food, and they constitute the majority of the volume and the value of the commercial starter cultures. The primary activity of the culture in food fermentation is to convert carbohydrates to desired metabolites as alcohol, acetic acid, lactic acid or CO2. Alcohol and organic acids are good natural preservatives, but also appreciated in their own right in the fermented product. The CO2 produced by some cultures contributes the gas needed to rise the dough. The cultures used in food fermentations are, however, also contributing by “secondary” reactions to the formation of flavor and texture. This secondary contribution can often be responsible for the difference between products of different brands, and thereby contribute significantly to the value of the product. Spontaneous sourdough fermentation is one of the oldest cereal fermentations known in mankind. Its main function was to leaven the dough to produce a more gaseous dough piece and as such a more aerated bread. In a later stage, beer yeast was used for dough leavening. Currently, researchers are still further elucidating the wide strain variety and the strain interactions using state-of-the-art analytical methods (Kulp and Lorenz, 2003).

Microflora of sourdough

Lactic Acid Bacteria (LAB) are constituted of a heterogeneous group of Gram-positive bacteria with a strictly fermentative metabolism from which lactic acid is the key metabolite. The natural habitat of these organisms includes humans, animals and plants. Their long history of safe use (Holzapfel et al., 2001), commonly referred to as the GRAS (Generally Recognized As Safe) status, combined with a variety of interesting metabolic characteristics have led to a wide range of industrial applications, i.e., Flavor, texture and preservative qualities of many fermented foods such as cheese, yoghurt, sausages, sourdough breads and silage (Wood, 1998). Genera of LAB
identified from sourdoughs are *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*, but the majority belongs to the genus *Lactobacillus*. The content of lactic and acetic acid is considered to be an important factor in the overall flavour perception during bread consumption. Homofermentative LAB are able to convert hexoses almost completely into lactic acid (>85%), whereas heterofermentative LAB degrade hexoses into lactic acid, acetic acid/ethanol, and CO₂. Besides the type of starter culture, also the temperature influences the ratio of lactic acid to acetic acid (Spicher and Rabe, 1980). Heterofermentative LAB also produce both, lactic and acetic acid, from pentoses (Hammes and Vogel, 1995). Several species of yeasts are also found in sourdoughs; *Saccharomyces cerevisiae* is frequently present or added. In particular, *S. exiguo*, *Torulopsis holmi*, *Candida krusei*, *Pichia norvegensis* and *Hansenula anomala* are generally found in yeast genera from the sourdoughs (Gobbetti et al., 1994).

**Proteolysis**

Lactic acid bacteria contribute to overall proteolysis during sourdough fermentation by creating optimum conditions for activity of cereal proteinases. Furthermore, lactic acid bacteria with high proteolytic activity contribute to the hydrolysis of wheat proteins in a strain-specific manner (Di Gagno et al., 2003). Generally, sourdough fermentation with lactic acid bacteria results in an increase of amino acid concentrations during fermentation, whereas dough fermentation with yeast reduces the concentration of free amino acids. The level of individual amino acids in wheat doughs depend on the pH level of the dough, fermentation time and the consumption of amino acids by the fermentative microflora (Thiele et al., 2002). In wheat sourdoughs, *Lb. brevis linderi*, *Lb. sanfranciscencis*, *Lb. brevis* and *Lb. plantarum* have been reported to increase the levels of aliphatic, dicarboxylic, and hydroxyl amino acids (Collar et al., 1991, Gobbetti et al. 1994). The yeasts *S. cerevisiae* and *S. exiguo* decrease the total level of amino acid in a similar way, the latter being more effective in amino acid removal from the dough (Spicher and Nierle 1984). The combination of yeast and lactic acid bacteria shows intermediate values for total amino acid levels. The estimated content of free peptides of sourdough with *Lb. brevis* or *Lb. plantarum* is lower than the estimated amount of amino acids. Reactivity of peptides is higher during fermentation in comparison to amino acids, and both of the above-mentioned strains reduce the content of peptides during fermentation, especially if *S. cerevisiae* is associated with these LAB (Mascaros et al., 1994). The proteolytic activity of wheat sourdough depends on the microbial starter and the processing conditions. For wheat sours, the extraction rate of flour and the fermentation temperature have been reported to be the main factors with positive influence on the level of free amino acids and on the accumulation of hydrophobic and basic amino acids. Dough yield has also been reported to influence the level of amino acids; soft doughs contain a lower amount of amino acids (Martinez-Anaya 2003).

**Starch digestibility**

Food-related diseases, such as obesity and type 2 diabetes, are becoming a tremendous threat to the health. White wheat bread, most rice products, potato and many breakfast cereals are examples of common starchy foods producing high glycaemic responses. The GI of food is a ranking of foods based on their immediate effect on blood glucose levels (Foster-Powell et al., 2002). Sourdough has great potential to modify the macromolecules in the dough, the most well known examples being the ability of sourdoughs to reduce digestibility of starch (Liljeberg et al., 1995; Ostman, Nilsson et al., 2002). Organic acids also play a role in the postprandial glyceamic responses. They may be present in the raw foods, produced upon fermentation processing (such as sourdough fermentation) or added, as in the case of pickled food. Certain acids, such as acetic, propionic, and lactic acid have the ability to lower the postprandial blood glucose and insulin responses, when included in bread meals. In the case of acetic acid added to bread meals, both Brighenti et al. (1995) and Liljeberg and Bjorck (1998) have shown blood glucose lowering effects. The presence of lactic acid in bread, either added or formed during sourdough fermentation, has also been reported to reduce acute glycemic and/or insulinemic responses (Liljeberg et al., 1995). However, the lowering of glycemic and insulinemic responses of breads with added lactic acid could not be attributed to a reduced gastric emptying rate (Liljeberg and Bjorck, 1996). The role of a reduced rate of starch digestion in lowering glycemia in bread containing lactic acid was confirmed by Ostman et al. (2002). Furthermore, their work suggested that the presence of lactic acid during heat treatment promotes
interactions between starch and gluten, hence reducing starch bioavailability.

**Volatile compounds**

The generation of volatiles in sourdoughs is clearly influenced by the activity of the LAB and the sourdough yeasts. Generally, LAB are mostly responsible for the acidification. Both microorganisms are able to liberate aroma precursors, such as free amino acids, and it has been previously shown that the concentrations of free amino acids are increased significantly during sourdough fermentation (Hansen *et al*., 1989; Kratochvil and Holas, 1983). The key degradation reaction of amino acids during dough fermentation is the Ehrlich pathway leading to aldehydes or the corresponding alcohols, respectively (Schieberle, 1996). The content of lactic and acetic acid is considered to be an important factor in the overall flavour perception during bread consumption. Homofermentative LAB are able to convert hexoses almost completely into lactic acid (≥ 85%), whereas heterofermentative LAB degrade hexoses into lactic acid, acetic acid/ethanol, and CO2. Besides the type of starter culture, also the temperature influences the ratio of lactic acid to acetic acid (Spicher and Rabe, 1980). Heterofermentative LAB also produce both, lactic and acetic acid, from pentoses (Hammes and Vogel, 1995). In addition, the flour type used influences the amounts of both acids formed during fermentation, with fermented whole meal flour being higher compared to wheat flour (Gobbetti *et al*., 1995; Hansen and Hansen, 1994; Lund *et al*., 1989). The production of acids in sourdoughs also increased with increasing temperature due to a higher production of lactic acid, whereas the production of acetic acid was not influenced (Gobbetti *et al*., 1995; Hansen *et al*., 1989). The production of acetic acid in sourdoughs can be increased using heterofermentative cultures and added fructose as hydrogen acceptor (Gobbetti *et al*., 1995; Martinez-Anaya *et al*., 1994).

The LAB can also influence the production of certain volatile metabolites, e.g. the content of ethyl acetate was higher in firm wheat sourdoughs (Damiani *et al*., 1996) fermented with heterofermentative LAB compared to sourdoughs fermented with homofermentative LAB. On the other hand, the production of ethyl acetate was higher in fluid sourdoughs fermented with homofermentative LAB compared to firm sourdoughs. The flour type also influenced the content of ethyl acetate, which was higher in wheat sourdough made with high extraction flour and heterofermentative cultures compared to sourdough made with low extraction flour (Hansen and Hansen 1994). Also, the formation of the carbonyl compounds might be influenced by the sourdough microflora (Lund *et al*., 1989). As an example, the content of hexanal was very high in sourdoughs fermented with a noncommercial homofermentative culture. The content of diacetyl was higher in sourdoughs manufactured with homofermentative compared to heterofermentative cultures (Damiani *et al*., 1996; Hansen *et al*., 1989; Lund *et al*., 1989), and was also higher in the corresponding bread (Hansen & Hansen, 1996). Saccharomyces cerevisiae is the most frequent yeast species isolated from sourdough; however, other subspecies of Saccharomyces, Candida, Pichia and Hansenula have occasionally been isolated (Mantynen *et al*., 1999). Addition of yeasts (Saccharomyces and Hansenula genus) resulted in increased production of alcohols, esters and some carbonyl compounds as compared to sourdoughs without added yeast (Damiani *et al*., 1996; Hansen and Hansen, 1994). Especially, the amounts of ethanol, methylpropanol, 2- and 3-methylbutanol as well as ethyl acetate and diacetyl were considerably increased in sourdoughs with added yeast.

**Mineral bioavailability**

Wholemeal cereals are an important source of minerals such as K, P, Mg, or Zn, but unfortunately mineral utilization is limited by the presence of phytic acid (Lopez and Demigne, 1998). Wheat contains about 2–58 mg/g phytic acid, which is localised in the aleurome layer of the kernel as the magnesium–potassium salt (Garcia *et al*., 1999). Phytic acid is highly charged with six phosphate groups, and it forms insoluble complexes with dietary cations, thus hindering mineral bioavailability (Lopez *et al*., 1998). Reduction of phytic acid content during bread making depends on phytase action. As with other enzymatic reactions, various factors contribute to phytate degradation in doughs, including phytase activity, particle size of the meals, pH, temperature, water content, and fermentation time (Fretzdorff and Brummer, 1992). Phytate-degrading enzymes exist in cereals, yeast and lactic acid bacteria isolated from sourdoughs (Lopez *et al*., 2000; Shirai *et al*., 1994; Turk *et al*., 2000). Reduction of phytic acid content in different bread types may vary between 13 and
100%, the highest levels of phytic acid obtained with unleavened breads (Lopez et al., 2001). In general, low pH favours degradation of phytic acid, optimal pH value for hydrolysis being 4.5 in wheat doughs according to Fretzdorff and Brummer (1992). Thus, use of sourdoughs or acidified sponges can be adjusted to improve mineral bioavailability by increasing phytic acid hydrolysis. In the recent work of Lopez et al. (2001), the influence of yeast fermentation, and sourdough fermentation without and with yeast, were compared on degradation of phytic acid. Their results show that both types of sourdough fermentation reduces phytic acid content up to 62%, whereas conventional yeast fermentation reduced it only by 38%. Furthermore, acidification formed during sourdough fermentation also increased magnesium and phosphorus solubility with 20–30%. This effect was even more pronounced if the bran fraction of wheat (rich in phytic acid) was fermented with lactic acid bacteria; the percentage of phytic acid breakdown was near 90% (Lopez et al., 2001).

Inhibitory role

The antimicrobial activity of LAB is due to the production of organic acids (in particular, lactic acid and acetic acid), carbon dioxide, ethanol, hydrogen peroxide, and diacetyl. The inhibition, however, can also be caused by bacteriocins that are low molecular-mass peptides, or proteins, with a bactericidal or bacteriostatic mode of action (De Vuyst and Vandamme, 1994). The inhibitory spectrum of some bacteriocins also includes food spoilage and/or food borne pathogenic microorganisms. Those bacteriocins may thus contribute to the competitiveness of the producing strain in the fermented food ecosystem (Caplice and Fitzgerald, 1999). The use of bacteriocin producing LAB starter cultures or co-cultures in the fermentation of cereals is currently under investigation. Even under stringent conditions of production, bread can become contaminated with moulds or bacteria such as Bacillus subtilis and clostridia that subsequently grow and spoil the product. To avoid outgrowth of these contaminating microorganisms, addition of organic acids or approximately 15% sourdough to the common dough recipe is performed (Voysey and Hammond, 1993). Sourdough addition is the most promising procedure to preserve bread from spoilage, since it is in agreement with the consumer demand for natural and additive-free food products (Rosenquist and Hansen, 1998).

In general, LAB bacteriocins tend to be active against a wide range of mostly closely related Gram-positive bacteria (Jack et al., 1995). Gram negative bacteria are generally insensitive to bacteriocins from LAB strains because of their outer membrane providing them with a permeability barrier. The sensitivity of Gram-negative bacteria can be increased by sublethal injury of the cells, using for instance high hydrostatic pressure and pulsed electric field as nonthermal methods of preservation (Capliche and Fitzgerald, 1999). In addition, food grade chelating agents such as ethylene-diamine-tetra-acetic acid (EDTA) and citrate can be used to bind magnesium ions in the lipopolysaccharide outer layer of Gram-negative bacteria to render them susceptible to bacteriocins (Holzapfel et al., 1995). Yeasts and fungi are not inhibited by LAB bacteriocins (De Vuyst and Vandamme, 1994b). The use of LAB bacteriocins or bacteriocin-producing sourdough LAB strains that are active against Bacillus species can be advantageous, since these species can cause spoilage of bread by rope formation, and may constitute a health risk. Rope formation occurs principally in wheat breads that have not been acidified, or in breads with high concentrations of sugar, fat, or fruits (Beuchat, 1997). The acidification and production of acetic acid by heterofermentative lactobacilli in sourdough delay growth of B. subtilis in bread, provided that the raw material and technological pre-fermentation conditions are optimal (Ganzle, 1998).

Conclusions

Sourdough has proven useful in improving dough properties, bread texture and flavor, delays the staling process and prevents bread from spoilage. Texture improvement is especially important in production of gluten-free bread, where the demand for good texture is especially challenging. Specific modifications in baked product texture can be achieved by development of new sourdough cultures, and by optimizing acidity and interactions with grain components. The production of prebiotic oligosaccharides by sourdough lactic acid bacteria also is an interesting possibility. Sourdough has also shown useful in production of breads with slow starch digestibility and hence low glyceamic responses. With the knowledge about the genetics

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and the physiology of lactic acid bacteria, it is possible to engineer starters for a variety of purposes
where a suitable starter culture cannot easily be found in nature. More applications are expected to fully
utilize the potential of sourdough in this respect.

References


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