Sourdough and cereal fermentation in a nutritional perspective

Kaisa Poutanen, Laura Flander, Kati Katina

VTT Technical Research Centre of Finland, POB 1000, FI-02044 VTT, Finland
University of Kuopio, Food and Health Research Centre, Department of Clinical Nutrition, POB 1627, FI-70211 Kuopio, Finland

ABSTRACT

Use of sourdough is of expanding interest for improvement of flavour, structure and stability of baked goods. Cereal fermentations also show significant potential in improvement and design of the nutritional quality and health effects of foods and ingredients. In addition to improving the sensory quality of whole grain, fibre-rich or gluten-free products, sourdough can also actively retard starch digestibility leading to low glycemic responses, modulate levels and bioaccessibility of bioactive compounds, and improve mineral bioavailability. Cereal fermentation may produce non-digestible polysaccharides, or modify accessibility of the grain fibre complex to gut microbiota. It has also been suggested that degradation of gluten may render bread better suitable for celiac persons.

The changes in cereal matrix potentially leading to improved nutritional quality are numerous. They include acid production, suggested to retard starch digestibility, and to adjust pH to a range which favours the action of certain endogenous enzymes, thus changing the bioavailability pattern of minerals and phytochemicals. This is especially beneficial in products rich in bran to deliver minerals and potentially protective compounds in the blood circulation. The action of enzymes during fermentation also causes hydrolysis and solubilisation of grain macromolecules, such as proteins and cell wall polysaccharides. This changes product texture, which may affect nutrient and non-nutrient absorption. New bioactive compounds, such as prebiotic oligosaccharides or other metabolites, may also be formed in cereal fermentations.

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1. Introduction

Cereal fermentation is one of the oldest biotechnological processes, dating back to ancient Egypt, where both beer and bread were produced by the help of yeasts and lactic acid bacteria. Spontaneous fermentation must have been used in the very early days, just activating the naturally occurring microbes in milled grains. In the more recent past, use of sourdough has already been more systematic, and microbial cultures have been developed and maintained by saving part of the ferment for further use. The first motives for use of fermentation in baking were leavening, flavour formation, and improved stability. Gradually, with development of industrial baking, the trend of using white wheat flour and baker's yeast became the major practice internationally. The art of sourdough fermentation is again increasingly recognized, and now development of specific cultures and control of fermentation process has become the practice. Their use in baking (Brümmner and Lorenz, 2003; Clarke and Arendt, 2005), and impact on bread texture (Arendt et al., 2007) and flavour (Ur-Rehman et al., 2006) has been recently reviewed. At the same time, the concern and knowledge of the nutritional effects of cereal fermentation have increased, as reviewed previously by Katina et al. (2005).

During cereal fermentation, typically up to 24 h at moderate temperatures, the metabolic activity of the microorganisms present is in interaction with the grain constituents. Lactic acid bacteria produce lactic and acetic acids, lowering the pH typically below pH 5. Yeasts produce carbon dioxide and ethanol. 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 selectively enhances performance of certain enzymes, such as amylases, proteases, hemicellulases and phytases. The enzyme-induced changes, together with microbial metabolites, bring about the technological and nutritional effects of fermented cereal foods.

Sourdough fermentation can influence the nutritional quality by decreasing or increasing levels of compounds, and enhancing or retarding the bioavailability of nutrients (Fig. 1).

2. Improvement of the sensory quality of whole grain and high-fibre bread

There is increasing evidence that intake of whole grain foods and grain fibre protects against chronic diseases, such as type 2...
diabetes and cardiovascular disease (Mellen et al., 2008; de Munter et al., 2007). As consumer demand for healthy food is increasing, it is natural to develop cereal foods high in fibre and whole grain. Processing of these raw materials meets challenges with respect to the sensory quality of the resulting foods. On the other hand, in ancient times sourdough was typically used in processing of unrefined flour. The outer layers of grain are rich in dietary fibre, phytochemicals, vitamins, minerals, and also endogenous enzymes. The bran fraction therefore offers many possibilities for modification by the sourdough fermentation (Fig. 3).

Sourdough is a key element in traditional rye bread baking, where it contributes significantly to the processability, flavour and texture. Wholemeal rye bread could not be produced without the help of the fermentation process. Many of the observed changes, e.g. in dietary fibre degradation (Boskov Hansen et al., 2002) or solubilisation (Katina et al., 2007a), can be explained by the contribution of endogenous enzymes, especially xylanases. During rye sourdough fermentation, endogenous rye proteases, especially aspartic proteases, hydrolyze rye proteins, especially secalins. This generates amino acids and small peptides, which act as flavour precursors (Tuukkanen et al., 2005).

Fermentation of both wheat (Hassan et al., 2008; Salmenkallio-Marttila et al., 2001) and rye (Katina et al., 2007a) brans has been shown to be an efficient pre-treatment method of bran both in order to improve sensory quality of bran-containing bread, and to degrade antinutritive factors, such as phytic acid, in order to improve mineral bioavailability (Hassan et al., 2008; Lioger et al., 2007). Pre-fermentation of bran with yeast and lactic acid bacteria improved loaf volume (Fig. 2) and crumb softness during storage (Salmenkallio-Marttila et al., 2001; Katina et al., 2006) (Fig. 3).

3. Mineral bioavailability

Wholemeal foods provide a good source of minerals in the diet, including calcium, potassium, magnesium, iron, zinc and phosphorus. Especially magnesium has been suggested to contribute to the health protective value of wholemeal foods against type 2 diabetes. The bioavailability of minerals may, however, be limited...
due to the presence of phytate, myo-inositol hexaphosphate. Contents of 3–22 mg/g phytic acid have been reported in grains (Garcia-Estepa et al., 1999). Phytic acid is concentrated in the aleurone layers of grains and has a strong chelating capacity. By forming insoluble complexes with dietary cations, it impairs mineral absorption in humans. Phytases are able to dephosphorylate phytate, forming free inorganic phosphate and inositol phosphate esters, which have less capacity to influence mineral solubility and bioavailability.

Phytase activity is present in grain raw materials, as well as in yeasts and lactic acid bacteria. Phytase action is accelerated in the acidic environment produced in sourdough fermentation. The pH optimum of wheat phytase is pH 5.0, whereas that of yeast phytase is pH 3.5 (Türk et al., 1996). It has been stated that a moderate decrease of pH to 5.5 during sourdough fermentation is sufficient to reduce phytate content of whole-wheat flour by about 70% by the endogenous phytase present in the flour (Leenhardt et al., 2005). The result was stated to highlight the predominance of endogenous flour phytase activity over sourdough microflora phytase activity. Screening of 50 lactic acid bacteria strains isolated from sourdoughs did not reveal significant phytase production (Reale et al., 2007), whereas in some other studies sourdough-originated lactic acid bacteria have been reported to degrade phytic acid when offered as the only carbon source (Shirai et al., 1994; Lopez et al., 2000). It anyway seems obvious that acid production and lowering of pH is the major mechanism for LAB to improve mineral bioavailability.

On the other hand, commercial baker’s yeasts were shown to express phytase activity (Türk et al., 2000), and wide variation in phytase activity was detected in traditional sourdough starters containing yeast and lactic acid bacteria (Chaoui et al., 2003; Reale et al., 2004). High-phytase yeast strains have also been suggested to have potential as phytase carriers in the gastrointestinal tract (Haraldsson et al., 2005).

Enzymatic phytate degradation depends on many fermentation parameters: phytase activity present, particle size of the flour, acidity, temperature, time and water content (Harinder et al., 1998; De Angelis et al., 2003). Sourdough fermentation was shown to be effective in solubilising minerals in whole-wheat flours, but to be less effective with bran. Calcium and iron solubilisation during fermentation was effective in finely milled bran particle size, whereas no solubilisation was detected in course bran (Lioger et al., 2007).

Lopez et al. (2001) showed that pre-fermentation of bran with lactic acid bacteria increased phytate breakdown (up to 90%) and increased magnesium and phosphorus solubility. Absorption of zinc, magnesium, and iron was also higher in rats fed sourdough baked bread (Lopez et al., 2003).

4. Levels and stability of vitamins and bioactive compounds

Cereal foods have for long been known to be and important source of vitamins, such as thiamine, vitamin E and folates. Recently the knowledge of also other biologically active compounds in the grain has increased substantially, as these have been suggested to be among the factors contributing to the protective properties of whole grain foods (Slavin, 2003). The outer layers of grains contain much higher levels of phytochemicals, such as phenolic acids, alkylresorcinols, lignans, phytosterols, tocols, and folate than the inner parts (Liukkonen et al., 2003; Mattila et al., 2005). The varietal differences of these compounds in European wheat, rye, oat and barley cultivars was just analysed, and the results show promise in terms of developing varieties with optimised levels (Ward et al., 2008). Processing may decrease or increase the levels, and also modify the bioavailability of these compounds as reviewed by Slavin et al. (2000), and for the phenolic compounds of rye reviewed just recently by Bondia Pons et al. (2009).

The results so far about the effects of sourdough and cereal fermentation are scarce, but to a large extent show that this type of bioprocessing would enhance delivery of these compounds in the human circulation. Yeast fermentation has repeatedly been shown to increase the folate content in the baking process of both wheat (Karihuuto et al., 2004) and rye (Liukkonen et al., 2003; Karihuuto et al., 2004, 2006; Katina et al., 2007a). In rye fermentation the levels of folates more than doubled (Liukkonen et al., 2003). Karihuuto et al. (2006) compared the ability of different yeasts and lactic acid bacteria to effect the folate content in a rye sourdough, and concluded that the effects of sourdough bacteria are minimal.
but the synthesis of folate by yeast can increase the content over three-fold in the best case.

The content of thiamine has also been reported to decrease in the baking process, more in wheat than in rye baking (Martinez-Villaluenga et al., 2009), but to increase during yeast fermentation, especially after long fermentation time (Ternes and Freund, 1988; Batifoulier et al., 2005). The fermentation step can thus affect the overall retention of vitamins in the baking process. A short baking process was shown to decrease also the content of vitamin B1 in whole-wheat baking, but a prolonged yeast or sourdough fermentation maintained it. Whole-wheat breadmaking with yeast (from kneading to final bread), with long fermentations, resulted in a 30% enrichment in riboflavin. The use of mixed fermentation conditions (yeast plus sourdough) did not have a synergistic effect on B vitamin levels (Batifoulier et al., 2005). Losses have been observed for vitamin E during sourdough preparation and dough making (Wennermark and Jägerstad, 1992), and also Liukkonen et al. (2003) observed reduction in tocopherol and tocotrienol content. This might be due to sensitivity to contact with air.

Fermentation has been shown to increase the antioxidant activity (DPPH radical scavenging activity) in the methanol extracted fraction of rye sourdough, concurrently with increased levels of easily extractable phenolic compounds (Liukkonen et al., 2003, Table 1). Fermentation of rye bran with yeast was also shown to increase the level of free ferulic acid (Katina et al., 2007a). The antioxidant capacity of traditional rye breads baked with sourdough has been shown to be clearly higher than of common white wheat bread; the highest values reported for breads made with wholemeal flour (Michalska et al., 2007; Martinez-Villaluenga et al., 2009). Very recently, it was shown that wheat bran bioprocessed with yeast fermentation in combination with cell wall hydrolytic enzymes increased the in vitro bioaccessibility of phenolic compounds as well as the colonic end metabolite 3-phenylpropionic in breads (Mateo Anson et al., in press).

### 5. Influence of sourdough on starch digestibility

Dietary carbohydrate represents a major source of plasma glucose. An increase in the amount of rapidly digestible carbohydrate in the diet increases blood glucose levels, particularly in the postprandial period. The major carbohydrate sources in a Western diet contain rapidly digestible starch. Consequently, many common starchy foods like bakery goods, breakfast cereals, potato products and snacks produce high glycemic responses. There are strong indications that the large amounts of rapidly available glucose derived from starch and free sugars in the modern diet (foods with high glycemic index, GI, and high insulin index, II) lead to periodic elevated plasma glucose and insulin concentrations that are detrimental to health (Barclay et al., 2008).

Macro-and microstructure of cereal foods has profound influence on the digestibility of starch. Especially, the characteristics of starch per se are of crucial importance for glucose response. Amylose-rich starches are more resistant to amylolysis than waxy or normal starches. In vitro, native starches are hydrolyzed very slowly, and to a limited extent, by amylases (Björk et al., 1994). As a result of gelatinisation during processing, the in vitro rate of amylolysis increases dramatically (Lauro et al., 2000). Thus, the more gelatinised starch is, the more rapidly it will be digested (Östman, 2003). In many common starchy foods, such as in regular white wheat bread, starch is highly gelatinised and product structure is very porous, resulting in rapid degradation of starch in small intestine and very rapid rise of blood glucose level (high GI).

The means to slower starch digestibility in wheat flour based products such as bread, biscuits and breakfast cereals are rare, if the addition of high amount of intact kernels is excluded due to resulting inferior product quality and consumer preferences. For wheat bread, the use of pre-fermentation technology (sourdough) or the addition of soluble fibres are in a recent review the only suggested means to reduce GI (Fardet et al., 2006).

The fermentation of wheat and rye flour matrix with lactic acid bacteria (sourdough process) has been shown to lower GI of wholemeal barley bread (Liljeberg et al., 1995; Östman, 2003) and wheat bread (De Angelis et al., 2006; Maioli et al., 2008), and insulin index (II) of rye breads with varying fibre content (Juntunen et al., 2003). Several mechanisms have been proposed for the ability of sourdough processing to reduce starch digestibility. The effect is assumed to be mainly due to formation of organic acids, especially lactic acid, during fermentation. The physiological mechanisms for the acute effects of acids appear to vary; whereas lactic acid lowers the rate of starch digestion in bread (Liljeberg et al., 1995), acetic and propionic acids appear instead to prolong the gastric emptying rate (Liljeberg and Björck, 1998). Chemical changes taking place during sourdough fermentation have been postulated to diminish the degree of starch gelatinisation (Östman, 2003) which would partly explain lower digestibility of sourdough fermented cereal foods.

At the product level, tissue integrity, porosity and structure of starch are important characteristics influencing glycemic response. Rye breads baked from wholemeal or white rye flour with very different fibre contents produced lower insulin responses than white wheat bread, when the food portion size was standardised to provide 50 g of starch (Juntunen et al., 2003). Both rye bread types were baked with a sourdough process and with 40% of a total amount of rye flour being pre-fermented before incorporating into the dough. The results suggested that with all rye breads, regardless of bran content, less insulin was needed to regulate blood sugar from the same amount of starch in comparison to normal wheat bread. The influence is probably due to the more rigid and less porous structure of rye bread, and due to the presence of organic acid formed during sourdough fermentation (Autio et al., 2003).

There may be also other mechanisms for the sourdough to regulate GI/II of the products. For example, pH-dependent proteolysis generally occurs during sourdough fermentation (Gänzle et al., 2008) producing significant amount of peptides and amino acids into the sourdough. Resulting increased concentration of amino acids and peptides in fermented cereals may have a role in regulating glucose metabolism (Nilsson et al., 2007). Furthermore, recent results demonstrate that sourdough fermentation increases the amount of free phenolic compounds (Katina et al., 2007a), which may also have an impact on lowering the Gl/II (Solomon and Blannin, 2007).

Use of sourdough is, however, a challenging technology for lowering of Gl/II due to the low pH (pH 4.1–4.5) required. Considering wheat based products, this pH is usually too low to be acceptable for consumers, and means for enhancing the efficacy of fermentation while maintaining higher pH levels would be desirable.

### Table 1

<table>
<thead>
<tr>
<th>Phenolic acids</th>
<th>After germination</th>
<th>After fermentation</th>
<th>After combined germination and fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free</td>
<td>1.4</td>
<td>3.6</td>
<td>9.9</td>
</tr>
<tr>
<td>Esterified</td>
<td>2.1</td>
<td>1.4</td>
<td>2.8</td>
</tr>
<tr>
<td>Glycosidc</td>
<td>1.6</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Bound</td>
<td>1.4</td>
<td>1.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Alk(en)lysresinosol</td>
<td>1.0</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Lignans</td>
<td>1.8</td>
<td>1.3</td>
<td>3.7</td>
</tr>
</tbody>
</table>

* Native whole grain rye – 1.
6. Sourdough and celiac disease

Celiac disease is a chronic inflammatory disorder characterized by damage of the small intestinal mucosa caused by the gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamins) of barley and rye in genetically susceptible subjects (Mäki and Collin, 1997; Fasano and Catassi, 2001). The disease, increasingly diagnosed throughout the world, can only be controlled by maintaining a strictly gluten-free diet. Rice, maize, sorghum, millet, teff, buckwheat, amaranth, and quinoa are suitable for celiac patients, who often suffer also from lack of dietary fibre and non-efficient mineral absorption. Oat has slightly different prolamins (avenins), and thus has been recently approved as ingredient in gluten-free labelled products by EC (if the cross-contamination from wheat, barley and rye can be avoided and gluten content of the oat product remains <20 mg/kg) (European Commission, 2009).

In baking applications the absence of wheat gluten poses a challenge to maintain good sensory quality, especially bread structure and/or retention of softness during storage. 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 et al., 2006, 2007, 2008). Improved textural properties have been reported for sourdough-made sorghum breads (Schober et al., 2007). The use of exopolysaccharides (EPS)-producing strains has been one way of creating improved properties in sorghum sourdoughs (Schwab et al., 2008).

Sourdough has also been studied for gluten degradation to render it suitable for celiac persons. The degradation of the cereal proteins in wheat and rye sourdough fermentations is an acidity related phenomenon, which strongly affects the flavour and texture of bread. Acidification and the reduction of disulfide bonds of gluten by heterofermentative lactobacilli increase the activity of cereal proteases and substrate accessibility; amino acids are accumulated by action of strain-specific intracellular peptidases of lactobacilli. Germinated cereals or other proteases enable an extensive degradation of proteins in sourdoughs in fermentation protocols that may be used to develop new products for individuals with gluten intolerance (Gänzle et al., 2008).

Thus, proteolysis by lactic acid bacteria has been suggested as a new tool for food processing for celiac persons (De Angelis et al., 2006; Di Gagno et al., 2008; Rizzello et al., 2007). The use of selected sourdough cultures to eliminate risks of contamination by gluten and to enhance the nutritional properties of gluten-free bread was highlighted by Di Gagno et al. (2008). The initial gluten contamination of 400 ppm in a gluten-free recipe was decreased to below 20 ppm using sourdough culture. Longtime fermentation of dough by selected lactic acid bacteria was also shown to be a potential tool to decrease the risk of rye contamination of gluten-free products for celiac patients (De Angelis et al., 2006).

Loponen et al. (2007, 2009) used germinated wheat or rye sourdough to degrade wheat and rye prolamins effectively. The use of cereals with low prolamin contents as a part of bread recipes could improve and diversify the flavour and nutritional properties of low-gluten breads and increase the assortment of cereal products that are designed for gluten-sensitive people. However, because the accuracy of prolamin analyses is unclear in terms of celiac safety, evidence on the clinical safety of products from such technologies is necessary before using such baking improvers in industrial practice.

7. Sourdough and gut health

Gut microbiota complements human nutrient metabolism, and contributes significantly in maintaining an extensive and highly active immune system. Recent evidence suggests that microbial perturbations play a role in development of metabolic diseases. Sourdough fermentation may influence gut heath by several mechanisms: 1) modulating dietary fibre complex and its subsequent fermentation pattern, 2) producing exopolysaccharides with prebiotic properties and 3) possibly providing metabolites from LAB fermentation which influence gut microbiota.

Interactions between dietary factors, gut microbiota, and host metabolism are increasingly demonstrated to be important for maintaining homeostasis and health (Cani and Delzenne, 2007), but research into the role of fibre structure and phytochemicals in gut microbiota mediated signalling is in its early phases. The physiological effects of dietary fibre are dependent on its physico-chemical properties, which are mainly influenced by particle size, cell wall architecture, solubility, degree of polymerisation and substitution, distribution of side chains and degree of cross-linking of the polymers. Recent results demonstrate the efficacy of fermentation to increase the bioavailability of fibre related compounds such as free ferulic acid. In wheat bran, ferulic acid is the most abundant phenolic compound. Ferulic acid is a structural component in cell walls, cross-linking cell wall polysaccharides. Since most of ferulic acid is covalently bound to the cell wall structures, its bioavailability in physiological conditions is suggested to be low. Recent results (Mateo Anson et al., in press; Napolitano et al., 2009) show that bioavailability of ferulic acid can be increased by processing of cereal bran and fibre with fermentation and enzyme treatments. We have also shown that the release of dietary fibre associated lignans and phenolic acids together with other phytochemicals of the aleurone layer of the rye grain may be modulated by fermentation (Katina et al., 2007a,b).

Sourdough-originated EPS also provide an opportunity to improve gut health. Certain lactic acid bacteria produce EPS, such as glucan, fructans and gluco- and fructo-oligosaccharides which have potential gut health promoting properties. Gut microbes metabolise dextran to propionic acid, which has been postulated to have several beneficial effects (Jann et al., 2006) such as reduced cholesterol and triglyceride levels, and increased insulin sensitivity. The levans produced by Lactobacillus sanfranciscensis have been shown to posses prebiotic properties (Korakli et al., 2002). Gluco-oligosaccharides (See et al., 2007) and fructo-oligosaccharides are potential EPS from sourdoughs to have prebiotic properties (Tiekking and Gänzle, 2005). Formation of oligo- and polysaccharides with prebiotic potential has been also shown by Lactobacillus reuteri LTH5448 and Weissella cibaria 10M in sorghum sourdoughs (Schwab et al., 2008).

The claimed health benefits of most of probiotic fermented foods are expressed either directly through the interaction of ingested live microorganisms, bacteria or yeast with the host (probiotic effect), or indirectly as a result of ingestion of microbial metabolites produced during the fermentation process (biogenic effect) (Stanton et al., 2005). Although still far from fully understood, several probiotic mechanisms of action have been proposed, including competitive exclusion, competition for nutrients and/or stimulation of immune response. The biogenic properties of fermented functional foods result from the microbial production of bioactive metabolites such as certain vitamins, bioactive peptides, organic acids or fatty acids during fermentation, which have shown to possess antihypertensive, antimicrobial and immunomodulatory properties in milk fermentations (Stanton et al., 2005). In addition, recent research interestingly suggests that cell wall components of Lactobacillus plantarum (strain also present in sourdoughs) stimulate immune response in the gut, and the bacterial cell does not necessarily have to be alive to yield such effects (Van Baaren et al., 2009). Thus, cereal fermentations have good potential to promote gut health in future applications, but research in this area is still at very early phases.
8. Future prospects

Sourdough is established technology in improving and diversifying the sensory quality of bread, and especially in whole grain-type baking it is finding good use. The concept of bran fermentation has also been introduced to assist in managing more bran in palatable form for high-fibre baked goods. Fermentation and acid production have been consistently shown to bring about improved mineral bioavailability. Sourdough and yeast fermentation may also increase the levels of bioactive compounds, but here more research is warranted. Sourdough baking is also consistently shown to deliver breads with slow starch digestibility and hence lower glyce- mic responses, and has shown promise in improving texture of gluten-free bread for celiac patients.

In the future, it can be anticipated that sourdough processing could be used to design foods with specific gut-mediated health effects, such as demonstrated changes in composition or activity of intestinal microbiota. The extracellular polysaccharides produced by lactic acid bacteria could act as selective or functional substrates for gut microbiota. Starter cultures themselves could possibly also bring probiotic-type properties in cereal foods, especially in those where no heat treatment is used. New bioactive metabolites could be produced during fermentation from the precursors present in the raw materials. Modification of the cereal matrix during fermentation could be tailored so as to increase the bioaccessibility of bioactive compounds. Production of bioactive peptides remains a yet quite unexplored potential, which could be accomplished by utilizing the proteolytic activity of the acidified cereal system.

As with other food processing, the challenge in fermenting cereal raw materials lies in the ability to combine good sensory quality with demonstrated nutritional and health benefits. Some of the mechanisms to improve and enhance the nutritional effects of fermented cereal systems described above are dependent on adjustment of the acidity for optimal action of the enzyme system present. Other mechanisms may be directly linked to other metabolites produced by yeast and lactic acid bacteria, and then the control of different metabolic routes in the fermenting organisms becomes a key issue. In any case, sourdough and cereal fermentations are a powerful and diversified tool for producing both sensory and nutritional quality attributes for many more foods than so far have been realised.

References


