

Sourdough Technology—A Traditional Way for Wholesome Foods: A Review

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Abstract: In the present era, consumers wish to have a wide range of foods that are nutritious and flavorful and have long shelf life without added preservatives. Sourdough is an important modern fermentation of cereal flours and water based upon an earlier spontaneous process. The sourdough microflora is dominated by lactic acid bacteria and, along with yeast, they play a key role in the fermentation of bread dough. Factors that affect the quality of sourdough are dough yield, temperature, type of starter culture, acidity of the medium, and the substrate. Sourdough is classified into 3 types (Types I, II, and III); the most widely used for commercial production is Type III. The sourdough fermentation has a number of beneficial effects that include prolonged shelf life, accelerated volume gain, delayed staling, improved bread flavor, and good nutritional value. Sourdough also improves sensory characteristics such as loaf volume, evenness of baking, color, aroma, taste, and texture of breads. Sourdough has been reported to contribute to extended shelf life by inhibiting spoilage bacteria and mold growth.

Introduction

Bread making is probably one of the oldest technologies known to mankind. Findings suggest that people of Babylon, Egypt, Greece, and Rome used bread as part of their diet long before the A.D. period. Bread is consumed in large quantity in the world in different types and forms depending on cultural habits. Flat breads are the oldest, most diverse, and most popular product in the world. It is estimated that over 1.8 billion people consume various kinds of flat breads all over the world. Bread products and their production techniques differ widely around the world. The objective of bread making is to convert cereal flours into attractive, palatable, and digestible food. The foremost quality characteristics of leavened wheat breads are high volume, soft and elastic crumb structure, good shelf life, and microbiological safety of the product (Cauvain 2003; Chavan and Jana 2008). However, fresh bread is a product with a short shelf life and during its storage, a number of chemical and physical alterations occur known as staling. As a result of these changes, bread quality deteriorates gradually as it loses its freshness and crispiness while crumb firmness and rigidity increase. The pleasant aroma vanishes and flavor assumes a stale feeling (Chavan and Jana 2008). Regardless of the type of technology, a bakery is currently using or willing to use, specific or tailor-made conditioning systems, and technology allows medium and large wholesale bakeries to produce high-quality goods by combining know-how on ingredients and on processing parameters. Additional technologies have appeared on the market in the early 1990s. While the opportunities for microwave baking of

(frozen) dough are still being explored, microwaves have entered the baking trade for defrosting and/or reheating purposes. Fresh products were predicted to and did assume a 4% decline in market share versus frozen products by 2006 (Chavan and Chavan 2010). Frozen bakery products are characterized by quick preparation time and affordable price and look and taste as if freshly made and homebaked (Yamauchi and others 2001; Giannou and others 2003).

The use of the sourdough process as a means of leavening is one of the oldest biotechnological processes in cereal food production. Sourdough bread is prepared from a mixture of flour and water that is fermented with lactic acid bacteria (LAB), mainly heterofermentative strains, elaborating lactic acid, and acetic acid in the mixture and hence causing a pleasant sour-tasting end product.

Role of Ingredients in Making Bread

Production of bread requires important ingredients such as flour, yeast, and water. Each ingredient used has its own significance both in conventional as well as in sourdough-based product manufacture. The roles of such ingredients are highlighted below:

Flour

Wheat-based foods are major source of nutrients in many regions of the world (Fincher and Stone 1986; Hoskeny and others 1988). Flour is the most important ingredient in bread making because it modulates the specific characteristics of bakery products. It consists of protein, starch and other carbohydrates, ash, fibers, lipids, water, and small amounts of vitamins, minerals, and enzymes. Wheat flour is the most common flour used. Refining of flour greatly affects the protein content as it decreases from 14.2% at 100% extraction to 12.7% at 66% extraction of flour (Pedersen 1994). The 2 basic types of protein that wheat flour

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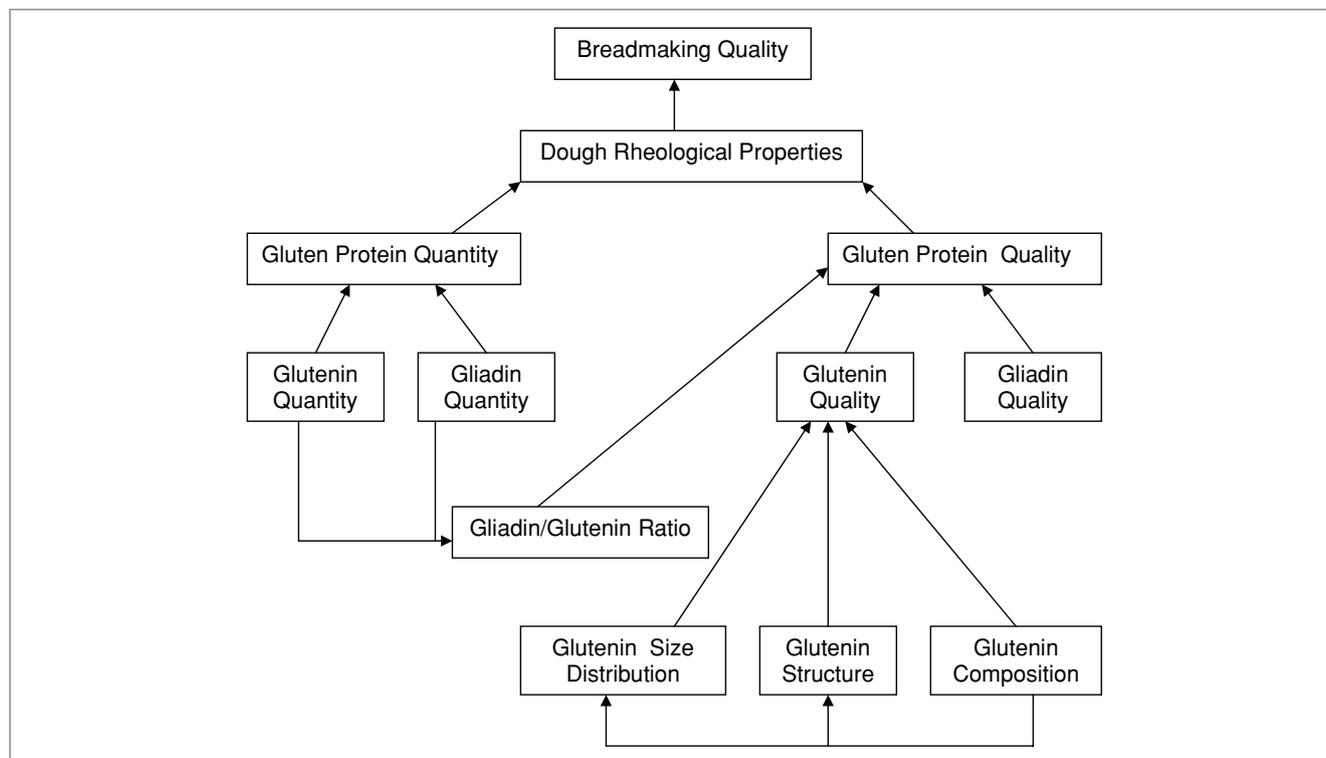


Figure 1—Factors governing bread-making quality and wheat dough rheological properties (adapted from Veraverbeke and Delcour 2002).

contains are gliadin and glutenin. When their mixture, known as gluten, is wetted, as during the preparation of dough, they form a cohesive and elastic network, which gives to wheat its functional properties (Giannou and others 2003; Chavan and Jana 2008). Based on primary structure, glutenin subunits have been divided into the high-molecular-weight (HMW) subunits (MW 67000 to 88000) and low-molecular-weight (LMW) subunits (MW 32000 to 35000). Native glutenins are composed of a backbone formed by HMW subunit polymers and of LMW subunit polymers branched off from HMW subunits. Noncovalent bonds, such as hydrogen bonds, ionic bonds, and hydrophobic bonds, are important for the aggregation of gliadins and glutenins and implicate structure and physical properties of dough. The quantity and quality of gluten proteins largely determine dough mixing requirements and sensitivity to overmixing. Furthermore, they determine the rheological properties of the optimally mixed dough (Figure 1) and as such contribute to the gas retention properties of the fermenting dough (Gan and others 1995). Gas retention properties, in turn, determine loaf volume and crumb structure of the resulting bread.

Yeast

Saccharomyces cerevisiae is the most common yeast used in bread making. Yeast cells metabolize fermentable sugars (glucose, fructose, sucrose, and maltose) under anaerobic conditions producing carbon dioxide (CO₂) as a waste product, which acts as a leavening agent and enhances dough volume (Giannou and others 2003; Chavan and Jana 2008).

Water

Water is necessary for the formation of dough and is responsible for its fluidity. It is used for the dissolution of salt and sugars and assists the dispersion of yeast cells. Water is needed for starch

and sucrose hydrolysis. It is important for starch gelatinization during baking and contributes to oven spring through vaporization (Giannou and others 2003; Chavan and Jana 2008).

Sugars

Sugars are normally used by yeast during the early stages of fermentation. Later more sugars are released for gas production by the action of enzymes in the flour. Sugars also act as antiplasticizers retarding pasting of native starch or function as antistaling ingredients inhibiting starch recrystallization (Giannou and others 2003; Chavan and Jana 2008).

Salt

Sodium chloride is considered as an ingredient with a functional role in the production of many bakery products. Salt strengthens the gluten, controls the action of yeast, and therefore controls the loaf volume. A small amount of salt in dough improves flavor and favors the action of amylases helping to maintain a supply of maltose as food for the yeast (Giannou and others 2003; Chavan and Jana 2008).

Lipids

Lipids can be used in bread making either in the form of fats or oils and are usually referred to as shortening. They are an optional ingredient in bread but can improve dough handling and crumb appearance and contribute to product flavor. Lipids also improve the keeping quality, softness, and moistness and contribute to bread texture (Giannou and others 2003; Chavan and Jana 2008).

Sourdough Processing

Spontaneous “sour” dough fermentation is one of the oldest cereal fermentations known to 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 (Spicher and Stephan 1999; Kulp and Lorenz 2003). In today's world, the first fermentation step, the bulk proof, is reduced or even excluded during the mechanization of the bakery. To catch up with the loss of flavors, some bakeries have developed or adapted brew systems. A part of the dough is fermented for several hours to develop a pleasant aroma that is known as sponge, brew, or polish. Hereafter, sponge is mixed with the rest of the ingredients into the final dough. With the next step in the evolution, to avoid or simplify this complex processing in the bakery, specialized companies now supply dried sourdoughs to the bakery industry. Further automation includes automated liquid or powder dosing systems, continuous or automated batch mixers, continuous belt lines and proofboxes, tunnel ovens, spiral coolers, automated slicing, packaging machines, and robot arms that place the final product on pallets. The sourdough fermentation is a traditional process for improving bread quality and producing different wheat and rye breads (Thiele and others 2002). At present, the sourdough is employed in the manufacture of breads, cakes, and crackers (Ottogalli and others 1996). The typical characteristic of sourdough is mainly due to its microflora, basically represented by LAB and yeasts. Due to its microbial life, such dough is metabolically active and can be reactivated. These microorganisms ensure acid production and leavening upon addition of flour and water. The mechanisms in sourdough are complex (Hammes and Gänzle 1998). Various flour characteristics and process parameters contribute to exercise very particular effects on the metabolic activity of the sourdough microflora. During fermentation, biochemical changes occur in the carbohydrate and protein components of the flour due to the action of microbial and indigenous enzymes (Spicher 1983).

Properties of Sourdough

The use of sourdough in wheat breads has gained popularity as a mean to improve the quality and flavor of wheat breads. A vast array of traditional products relies on the use of sourdough fermentation to yield baked goods with particular quality characteristics. Some examples include the well-known Italian products associated with *Christmas*, *Panettone*, which originated in Milan. San Francisco sourdough, French breads, and soda crackers are other examples of wheat products that rely on the process of souring. The sourdough fermentation affects dough rheology at 2 levels, in sourdough itself, and in bread dough-containing sourdough. In dough, fermentation decreases elasticity and viscosity, whereas the addition of sourdough to final bread dough results in less elastic and softer dough. The level of rheological changes taking place in these dough and its influences on bread quality can be controlled by adjusting fermentation time and the ash content of flour during the prefermentation process (Clarke and others 2004). Many inherent properties of sourdough rely on the metabolic activities of its resident LAB: lactic fermentation, proteolysis, and synthesis of volatile compounds, antimould, and antiropiness production is among the most important activities during sourdough fermentation (Hammes and Gänzle 1998; Gobetti and others 1999). Moreover, endogenous factors in cereal products (carbohydrates, nitrogen sources, minerals, lipids, and free fatty acids, and enzyme activities) and process parameters (temperature, dough yield [DY], oxygen, fermentation time, and number of sourdough propagation steps) markedly influence the microflora of sourdough and the features of leavened baked goods (Hammes and Gänzle 1998). Some of the factors are explained as follows:

Dough yield

Sourdough can vary in its consistency. The sourdough fermentation can be performed as firm dough or as a liquid suspension of flour in water. This proportion between flour and water is called the DY and is defined as:

$$\text{Dough yield} = (\text{amount of flour} + \text{amount of water}) \times 100 / \text{amount of flour}$$

The DY value of a sourdough will significantly influence the flavor profile of the sourdough. The firmer the sourdough (lower DY value), the more acetic acid is produced and the less lactic acid. The acidification rate is also influenced by the DY of a sourdough. The higher the DY, the faster the acidification will occur, most probably due to the better diffusion of the produced organic acids into the environment (Spicher and Stephan 1999).

Temperature

Temperature is the utmost important factor, as it influences DY more than acidification rate and also has an influence on the microbial composition of the sourdough. If backslopping is used where a part of the previous sourdough is used to inoculate the next fermentation, temperature plays a critical role because part of the microflora can be lost over the different sourdough refreshments if it is not controlled (Spicher and Stephan 1999). Optimum temperatures for the growth of *Lactobacilli* are 30 to 40° C depending on strain and for yeasts 25 to 27° C. In general, a higher temperature, a higher water content of sourdough, and the utilization of wholemeal flour enhance the production of acids in wheat sourdoughs (Brummer and Lorenz 1991).

Starter cultures

A 3rd parameter is the microflora used for the fermentation. Two main families can be distinguished: the heterofermentative and the homofermentative LAB. The flavor can easily be influenced changing the fermentation temperature as explained above. A commercially available sourdough starter commonly consists of mixtures of different LAB groups to assure good acidification and aromatization.

Titratable acidity and pH

The titratable acidity and pH of the dough are important during sourdough fermentation. In the initial phase, both acidity and pH remain constant, whereas during the intermediate phase, titratable acidity increases due to the presence of yeast. During the long-term fermentation phase, the yeast presence becomes negative and titratable acidity, and pH of the dough depends mainly on the LAB introduced into the system. The yeasts present in sourdough are only slightly influenced by lactic acid, but much more affected by acetic acid (Schulz 1972).

Substrate

The substrate, mainly flour, used for sourdough fermentation is another parameter that significantly influences the sourdough. Ash content is important to determine flour grade and extraction rate, since the ash content of the bran is about 20 times that of endosperm (Matz 1996). The ratio of bran to endosperm is higher in small kernels (Posner 2000). The bran fraction contains more minerals and micronutrients that are important for the growth of LAB. The ash also influences the buffering capacity of the sourdough system that makes possible to reach a higher total titratable activity. The falling number of the flour is an indicator for the enzymatic activity of the flour. The lower the value the more amylase

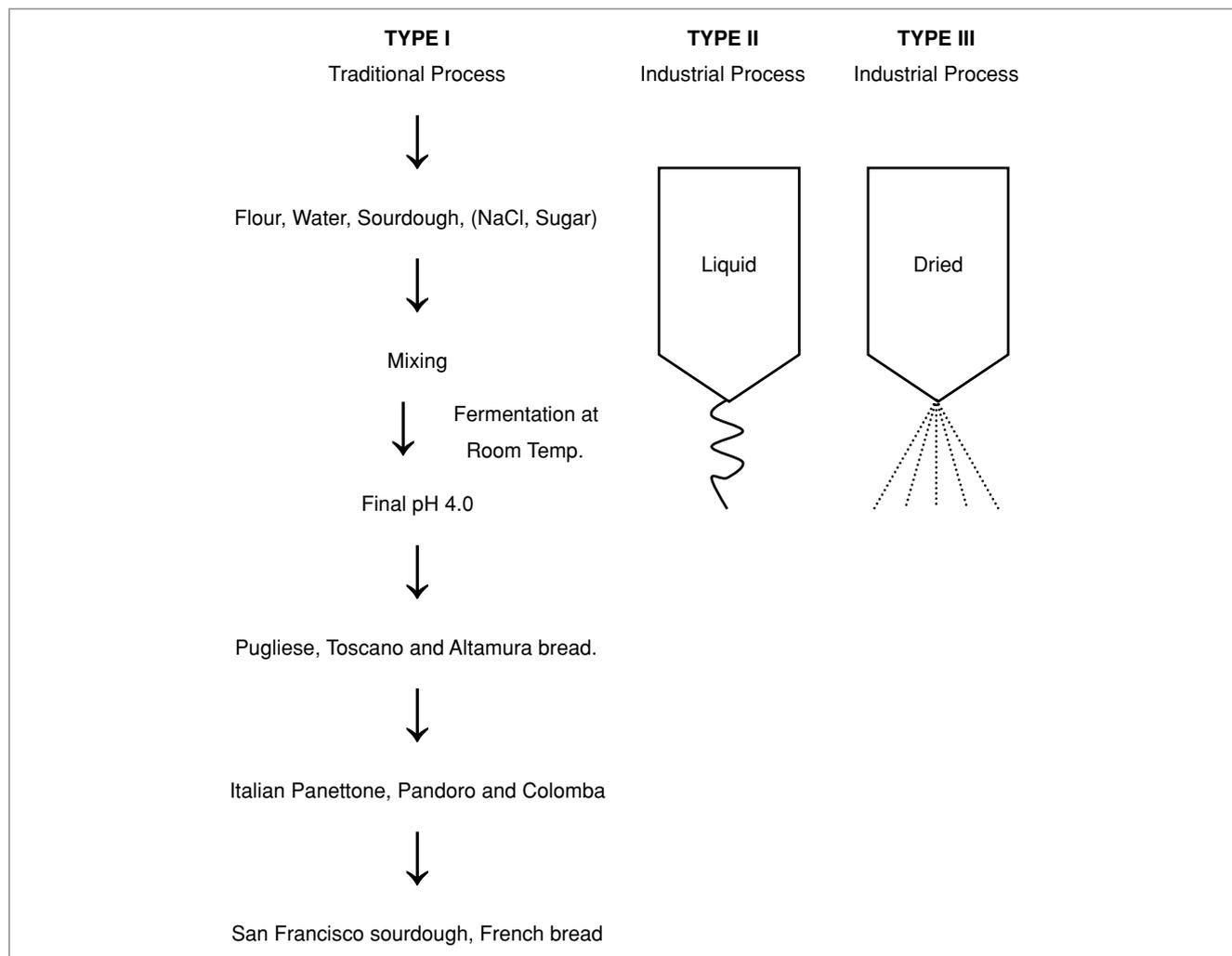


Figure 2–Scheme of sourdough production processes.

activity is present in the flour. At that moment, more free sugars will be available for the microflora to grow (Spicher and Stephan 1999).

Classification of Sourdough

Sourdoughs, on the basis of the technology applied, have been grouped into 3 types: Type I in which a sourdough that is restarted using a part of the previous fermentation. These are the traditional sourdoughs. Type II is an industrial type of sourdough using adapted strains to start fermentation. This sourdough can be liquid, so it is easily pumpable in an industrial bakery. Type III, sourdough, which can be dried, is often used by industrial bakeries since the quality is constant and there are no longer end-product variations due to the freshly produced sourdough (Figure 2). The Type III sourdoughs are the most convenient to introduce authentic bread taste into the nowadays high-tech bakery industry. In industry, a lot of Type III sourdoughs are available (Böcker and others 1995). Different drying techniques are used as well as liquid pasteurization, to achieve microbial stability. Spray-drying and drum-drying are the most commonly used drying techniques in Type III sourdough production.

The doughs of Types II and III require the addition of baker's yeast (*S. cerevisiae*) as leavening agent, whereas Type I sourdoughs do not require this addition. Sourdough LAB, consisting of ob-

ligate and facultative heterofermentative and obligate homofermentative species associated with Types I, II, and III sourdoughs. Lyophilized strains of *Lactobacillus delbrueckii*, *L. fructivorans*, *L. plantarum*, and *L. brevis* have been established as sourdough LAB (Hammes and Gänzle 1998). In contrast to the Type I sourdough starters, frequent inoculations of these strains are required as these are not well adapted to the cereal environment (Roecken and Voysey 1995). Due to the selective pressures that results from the environmental conditions of sourdough preparation, *L. sanfranciscensis* dominates Type I sourdough fermentations (Foschino and others 1999; Corsetti and others 2001).

In spray-drying, the liquid sourdough is pulverized in a hot air stream. The water content (about 90%) is evaporated, while the sourdough droplets are falling down in the hot air. Due to the presence of evaporating water in the falling hot droplets, the product itself is cooled down during the process thus avoiding browning of the powder. In the drum-drying technology, stainless steel cylinders are heated with steam. A thin film of product is spread over the cylinder and almost immediate evaporation occurs. The rest of the residence time of the semi-dry product on the drum will be used to allow Maillard reactions. Dependent on the temperature/time combination, the end sourdough can be more or less caramelized or toasted. A drum-dried Type III sourdough will not only add a sourdough flavor to the end product, but at

Table 1—*Lactobacillus* species generally associated with sourdough fermentation or found in fermented sourdough.

Obligately heterofermentative	Facultatively heterofermentative	Obligately homofermentative
<i>Lb. acidifarinae</i>	<i>Lb. plantarum</i>	<i>Lb. amylovorus</i>
<i>Lb. brevis</i>	<i>Lb. pentosus</i>	<i>Lb. acidophilus</i>
<i>Lb. buchneri</i>	<i>Lb. alimentarius</i>	<i>Lb. delbrueckii subsp. delbrueckii</i>
<i>Lb. fermentum</i>	<i>Lb. paralimentarius</i>	<i>Lb. farciminis</i>
<i>Lb. fructivorans</i>	<i>Lb. casei</i>	<i>Lb. mindensis</i>
<i>Lb. frumenti</i>		<i>Lb. crispatus</i>
<i>Lb. hilgardii</i>		<i>Lb. johnsonii</i>
<i>Lb. panis</i>		<i>Lb. amylolyticus</i>
<i>Lb. pontis</i>		
<i>Lb. reuteri</i>		
<i>Lb. rossiae</i>		
<i>Lb. sanfranciscensis</i>		
<i>Lb. siliginis</i>		
<i>Lb. spicheri</i>		
<i>Lb. zymae</i>		

the same time also some malted, caramelized flavor notes up to a toasted aroma. Using the previous stabilization processes, there is a loss of volatile flavor compounds during the evaporation of the water. A way to prevent this and to achieve more complete flavor properties is to keep the sourdough in a liquid form and to stabilize the sourdough by pasteurization or by cooling. Most volatile compounds remain present in the product. An advantage for the industrial application is the pumpability of such a product and also it is very accurate and has a constant quality at any time.

Sourdough Microbiota

Sourdoughs are very complex biological ecosystems because of the microbial composition and all interactive effects among the bread-making processes and ingredients (Gobbetti and others 1999). Cereal fermentations, namely sourdoughs, are dominated by specifically adapted LAB occurring at numbers above 10^8 CFU/g, which may be in coexistence or possibly in symbiosis with typical yeasts whose numbers are orders of magnitude lower (Gobbetti and others 1999; Vogel and others 2002). The majority of species regularly isolated from sourdough or used as sourdough starter belong, with only few exceptions, to 1 of the 4 genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella*. The highest number of different species (>23 species) is found in the genus *Lactobacillus*. There exists no discernible emphasis on any of the fermentation types classically dividing *Lactobacilli* in obligate homofermentative, facultative heterofermentative, and obligate heterofermentative strains (Table 1). The majority of yeasts found in sourdoughs have been allotted to the species *Candida milleri*, *C. holmii*, *S. exiguus*, and *S. cerevisiae* (Hammes and Gänzle 1998). Most of the yeast preparations often contain LAB, especially *Lactobacilli* rather than *Pediococcus*, *Lactococcus*, and *Leuconostoc* spp. (Jenson 1998), which contributes a little to the aroma development through acidification of the dough during the limited processing period (Rothe and Ruttloff 1983).

Yeasts are often associated with LAB in sourdough and the yeasts/LAB ratio is generally 1:100 (Gobbetti and others 1994; Ottogalli and others 1996). The yeasts found in sourdoughs belong to more than 20 species (Rossi 1996; Stolz 1999; Gullo and others 2003). Typical yeasts associated with LAB in sourdoughs are *S. exiguus*, *C. humilis* (formerly described as *C. milleri*), and *Issatchenkia orientalis* (*C. krusei*) (Spicher and Schroder 1978; Gobbetti and others 1995a, 1995b; Succi and others 2003). Other yeast species detected in sourdough ecosystem are *Pichia anomala* as *Hansenula anomala*, *Saturnispora saitoi* as *P. saitoi*, *Torulasporea del-*

brueckii, *Debaryomyces hansenii*, and *P. membranifaciens* (Gobbetti and others 1994; Foschino and Galli 1997; Succi and others 2003). The variability in the number and type of yeast species in dough are affected by many factors such as dough hydration, level and the type of cereal used, the leavening temperature, and the sourdough maintenance temperature (Gobbetti and others 1994).

Metabolic Pathways of LAB

The available carbohydrates in wheat flour are maltose followed by sucrose, glucose, and fructose, along with some trisaccharides such as maltotriose and raffinose. The glucose amount increases during fermentation, whereas sucrose decreases in the presence of yeasts due to the action of invertase. The yeasts present in sourdoughs are not able to ferment maltose, a sugar common in flour. However, yeasts cell can nevertheless develop because of glucose released into the medium by some LAB species, notably *L. sanfranciscensis*. Starting from glucose, homofermentative LAB mainly produce lactic acid through glycolysis (homolactic fermentation), while heterofermentative LAB also produce, besides lactic acid, CO₂, acetic acid, and/or ethanol (Figure 3) through the 6-phosphogluconate/phosphoketolase (6-PG/PK) pathway (heterolactic fermentation). Hexoses other than glucose enter these major pathways at the level of glucose-6-phosphate or fructose-6-phosphate after isomerization and/or phosphorylation (Axelsson 1999). Disaccharides are split by specific hydrolases and/or phosphohydrolases to monosaccharides that then enter the major pathways. Pentoses are phosphorylated and converted to ribulose-5-phosphate or xylulose-5-phosphate by epimerases or isomerases and subsequently metabolized through the lower half of the 6-PG/PK pathway (Axelsson 1999). Utilization of pentoses is not restricted to LAB species that possess a constitutive phosphoketolase, the key enzyme of the 6-PG/PK pathway (obligate heterofermentative); facultative heterofermentative LAB, which ferment hexose through glycolysis because they possess a constitutive fructose-1,6-diphosphate aldolase (key enzyme of glycolysis), ferment pentoses in the same way as obligate heterofermentative species. Under such circumstances, the phosphoketolase of facultative heterofermentative LAB is induced by the available pentose sugars (Hammes and Vogel 1995). Fermentation of pentoses results in the production of equimolar amounts of lactic and acetic acid; no CO₂ is formed, and since no dehydrogenation steps are necessary to reach the intermediate xylulose-5-phosphate, acetyl phosphate is used by acetate kinase in a substrate level phosphorylation step yielding acetate and adenosine triphosphate. Obligate homofermentative LAB do not ferment pentoses (Axelsson 1999). So far, some studies have been aimed at the sequencing of the complete genome of *Lactobacilli*, including *L. brevis* (Makarova and others 2006), *L. plantarum* (Kleerebezem and others 2004), and *Lb. reuteri* that are also found in sourdough.

The metabolic pathways of LAB that influence bread quality are coupled to the central carbon flux by the availability of cofactors influencing the cellular and environmental redox potential. Homo- and heterofermentative metabolism differ fundamentally with respect to the requirement for regeneration of reduced cofactors, reduced nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate. The utilization of cosubstrates, such as oxygen or fructose, as electron acceptors by obligate heterofermentative *Lactobacilli* is coupled to an increased production of acetate in dough. Based on the different metabolic requirements for cofactor regeneration, homo- and heterofermentative *Lactobacilli* exert divergent effects on the redox reactions in sourdough that influence bread quality beyond the formation of acetate.

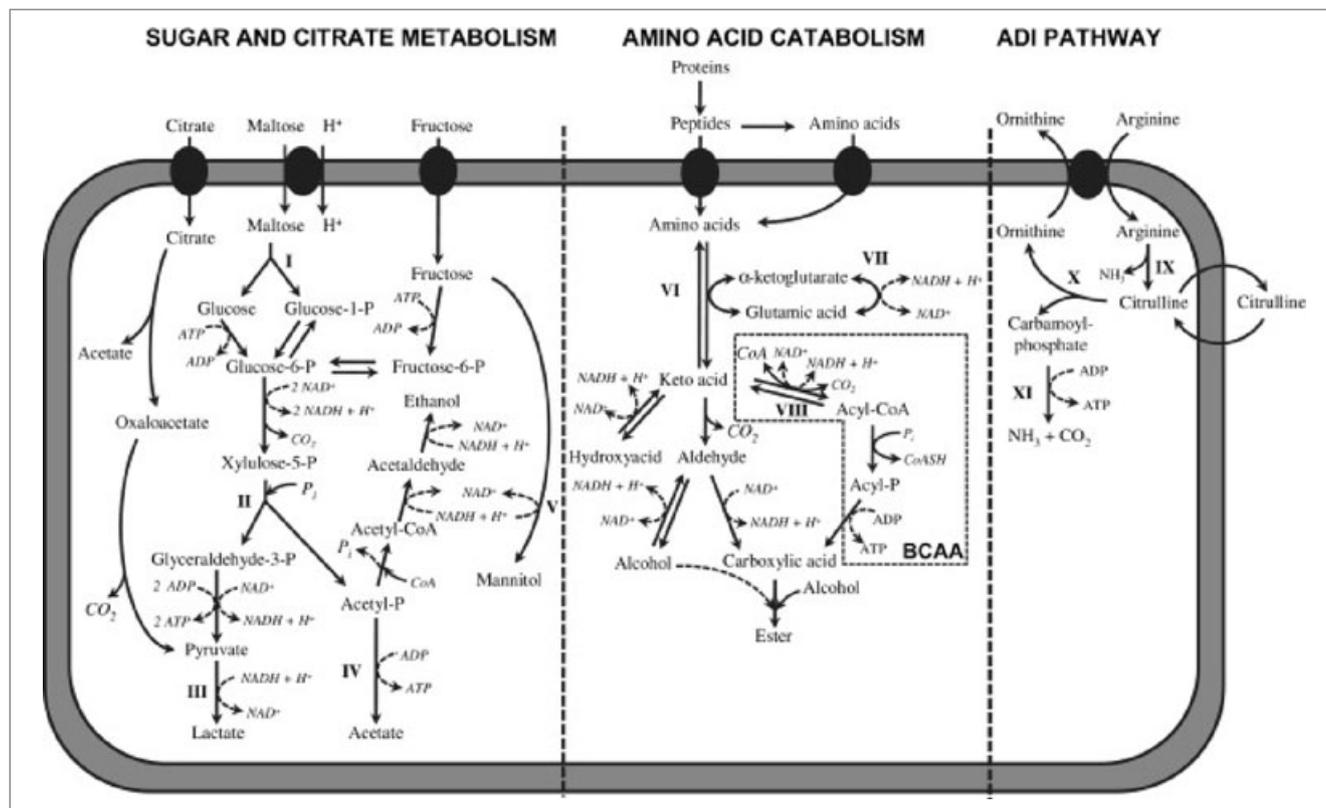


Figure 3—Pathways active in typical heterofermentative sourdough lactic acid bacteria (adapted from Liu and others 2008).

Metabolism of carbohydrates in LAB

Facultative (for example, *L. plantarum* and *L. alimentarius*) and obligate heterofermentative (for example, *L. sanfranciscensis* and *L. pontis*) LAB that use, respectively, the Embden-Meyerhof-Parnas and phosphogluconate pathways for hexose fermentation, are commonly found in sourdough. In addition to these main energy routes, the phenotypic responses to low and variable nutrient conditions involve the use of external acceptors of electrons, the hierarchical, and/or simultaneous use of various energy sources, often coupled with inducible uptake systems, and/or the interactions with endogenous and exogenous enzymes (Vogel and others 1999). During the phosphogluconate pathway, additional energy may be generated by the activity of acetate kinase that, in the presence of electron acceptors, allows the recycling of NAD^+ without the need of ethanol formation. Cofermentations are metabolic alternatives that enable sourdough LAB to use nonfermentable substrates, thus increasing their adaptability. A cometabolism of citrate and maltose or glucose was observed in *Lb. sanfranciscensis* (Gobbetti and Corsetti 1996). In the cofermentation process, pentoses were preferentially consumed instead of maltose (Gobbetti and others 1999). The formation of pyruvate and lactate may also derive from the obligatory use of a range of nonconventional substrates such as amino acids. Serine is deaminated to ammonia and pyruvate, which is reduced to lactate. Pyruvate is produced directly (from alanine) or indirectly (from aspartate) by transamination. Lactate degradation during sourdough fermentation may have an impact on sourdough flavor and texture due to the formation of acetate and CO_2 (Liu 2003).

Metabolism of nitrogen compounds in LAB

It has been well established that almost all LAB have multiple (4 to 14 amino acids) auxotrophy (Calderon and others 2003).

LAB, therefore, depend on proteolytic systems that allow degradation of proteins. The proteolytic system consists of an extracellularly located serine-proteinase, transport systems specific for di/tri-peptides and oligopeptides (>3 amino acid residues), and a multitude of intracellular peptidases. Generally, proteolytic enzymes (proteases) are grouped into proteinases and peptidases. Proteinases catalyze protein degradation into smaller peptide fractions; peptidases hydrolyze specific peptide bonds or completely break down peptides to amino acids. The proteolytic activity of wheat and rye flours is attributable mainly to aspartic proteinases and carboxypeptidase, with both these protease groups active under acidic conditions (Table 2).

The degradation of wheat and rye proteins is of crucial importance for bread flavor, volume, and texture. Gluten proteins, glutenins and gliadins, are the major storage proteins of the wheat grain. Gliadins are alcohol-soluble proteins of wheat grain and glutenins are soluble in dilute acids (Osborne 1907). Glutenins are highly polymeric proteins that are divided into HMW and LMW fractions (Shewry and Tarham 1994; Shewry and others 1996). The gliadins are monomeric as they contain only intramolecular disulfide bonds. Gliadins are grouped into α -, γ -, and ω -type gliadins based on their amino acid compositions. Alpha- and γ -gliadins, but not ω -gliadins contain cysteine residues (Shewry and Tatham 1997). The major storage proteins of rye are the alcohol-soluble secalins. The secalins are divided into γ -secalins, ω -secalins, and HMW secalins. It is generally observed that a limited extent of proteolysis during all sourdough fermentations beneficially improves the bread flavor without adverse effects on texture and volume (Thiele and others 2002). Amino acids and peptides affect the taste of fermented foods and, in particular, are important precursors for volatile flavor compounds. Amino acids serve as substrates for microbial conversions or are converted to

Table 2—The major protease groups of resting and germinated wheat grains (adapted from Loponen 2006).

Protease type	Wheat flour		Wheat malt			
	Aspartic proteinase	Serine carboxypeptidase II	Cysteine proteinases	Serine proteinases	Metallo proteinases	Serine carboxypeptidases I to V
Localization	Endosperm	Endosperm	Aleurone, germ	Endosperm, germ	Germ	Aleurone, germ, endosperm
pH range	3 to 4.5	4 to 6	4 to 6	5 to 7.5	n.d.	4 to 6
Specificity	Cleaves between hydrophobic amino acids	Liberates aromatic amino acids	Broad specificity	n.d.	n.d.	Broad specificity, praline specific
Activity against gluten proteins	+, Glutenins	Operates jointly with proteinases	+	+	n.d.	Operates jointly with proteinases

n.d. = not determined.

flavor compounds during baking; accordingly, a limited extent of proteolysis during fermentation improves bread flavor (Thiele and others 2002). The odor of breadcrumb is mainly determined by microbial fermentation products, whereas the taste and aroma products originating from thermal reactions dominate in the crust (Kirchhoff and Schieberle 2001).

The effect of acidification and of endogenous wheat proteinases, which have an optimum pH at 3.0 to 4.0, is considered important for proteolysis in the dough, especially for longtime sourdough fermentations. Strain-specific proteolytic activity of LAB may additionally contribute to proteolysis. Microbial acidification and the reduction of disulfide bonds in gluten proteins by heterofermentative *Lactobacilli* increase the solubility of gluten proteins and make them more susceptible for proteolytic degradation. The hydrolysis of peptides (secondary proteolysis) by sourdough *Lactobacilli* accumulates amino acids in dough in a strain-dependent manner, whereas yeasts decrease amino acids levels in dough. The metabolic activities during proteolysis are outlined in Figure 4 and 5.

Production of Exopolysaccharides (EPS)

The EPS are microbial polysaccharides secreted extracellularly, the amount and their structures depend on the particular microorganisms and the available carbon substrate (Korakli and others 2001). Cereal-associated *Lactobacilli* produce a large structural variety of EPS and oligosaccharides from sucrose through the activity of glycosyltransferases. These are produced by LAB during fermentation, one of the aspects of sourdough technology with the potential for replacement by hydrocolloids. These compounds, commonly called as gums, are used as texturizing, antistaling, or prebiotic additives in bread production (Tiekink and others 2003a, 2003b, 2003c). Orla-Jensen (1943) described EPS formation from sucrose by *Leuconostoc* spp., mesophilic *Lactobacilli*, and *pediococci* and indicated the role of EPS formation in the spoilage of apple cider and beer. Two classes of EPS from LAB can be distinguished, extracellularly synthesized homopolysaccharides and heteropolysaccharides (HePS) with irregular repeating units. The repeating units of HePS are composed of 3 to 8 carbohydrate moieties that are synthesized intracellularly from sugar nucleotide precursors (de Vuyst and others 2001). HePS application is currently limited to “ropy” dairy starter cultures employed to improve the texture of yogurt and other fermented milk products (Laws and Marshall 2001) and formation of HePS by cereal-associated *Lactobacilli* has hitherto not been described. Furthermore, fructan produced by 2 strains of *Lb. sanfranciscensis* has been shown to stimulate bifidobacterial growth (Dal Bello and others 2001), thus acting as a prebiotic or in this case as a bifidogenic factor. Modler (1994) reported that the application of prebiotics increases both

the occurrence and the number of fecal bifidobacteria that are among the most prominent bacteria found in the jejunum with widely accepted probiotic properties (Salminen and others 1998). Also, certain LAB strains are reported to possess health-promoting activities (Lee and Salminen 1995). Among such probiotic LAB strains are those that have been isolated from cereal-based fermented food being capable of adhering to human intestinal cells (Müller and others 1998). From this is clear that bifidogenic substrates produced by LAB (which are generally recognized as safe) and resistant to low pH (not degraded during fermentation) and high temperature (still available after baking) may be acceptable in foods as a possible prebiotic component of that food. Furthermore, species, such as *Lb. pontis*, *L. panis*, *L. mucosae*, *Lb. reuteri*, *L. oris*, *Lb. acidophilus* (Roos and others 2000; Simpson and others 2000; Vogel and others 2002), and recently *Lb. rossiae* (De Angelis and others 2006), have been found both in human and animal intestines and feces, as well as in sourdoughs, and their possible probiotic activity merits further investigation (Vogel and others 2002).

Volatile Metabolites in Sourdough

Taste and aroma smell, the flavor, are undoubtedly the most important attributes determining the quality of bread or baked cereals in general. Sourdough fermentation has a well-established role in improving flavor and structure of rye and wheat breads (Brunner and Lorenz 2003). In principle, the whole grain or fractions of cereal grain can be modified by sourdough fermentation to improve nutritional value or promote healthfulness of cereal foods. Wholemeal flour is rich in fiber, minerals, vitamins, and many phytochemicals such as phenolic compounds, sterols, tocopherols, and tocotrienols, lignans, and phytic acid. With sourdough processes, the mouthfeel and palatability of wholemeal bread can be improved without removing any nutritionally important components (Salmenkallio-Marttila and others 2001). Sourdough bread has a higher content of volatiles and, also, achieves higher scores in sensory tests compared to, for example, bread chemically acidified with lactic and acetic acid (Hansen and others 1989; Hansen and Hansen 1996). Some of the compounds present in bread have been shown to be related to the concentrations in the corresponding sourdoughs. As an example, the contents of methylpropanol (*iso*-butanol), 2- and 3-methylbutanol (*iso*-pentanols), ethyl acetate, and ethyl lactate in 3 bakery sourdoughs were clearly related to the amounts of these compounds in the sourdoughs (Hansen and Lund 1987; Lund and others 1987).

There are 2 categories of flavor compounds produced during sourdough fermentation. Nonvolatile compounds, including organic acids, produced by homo- (Gobbetti and others 1995a) and

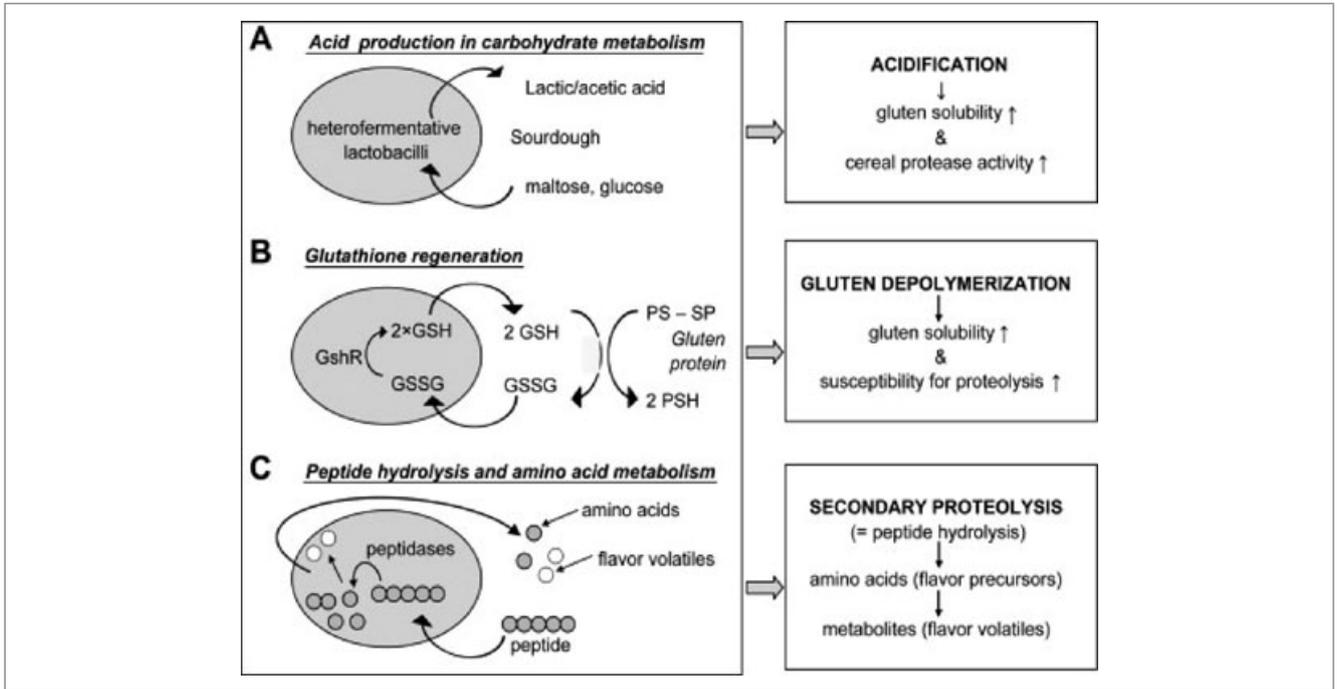


Figure 4–Contribution of lactic acid bacteria to proteolysis in sourdoughs.

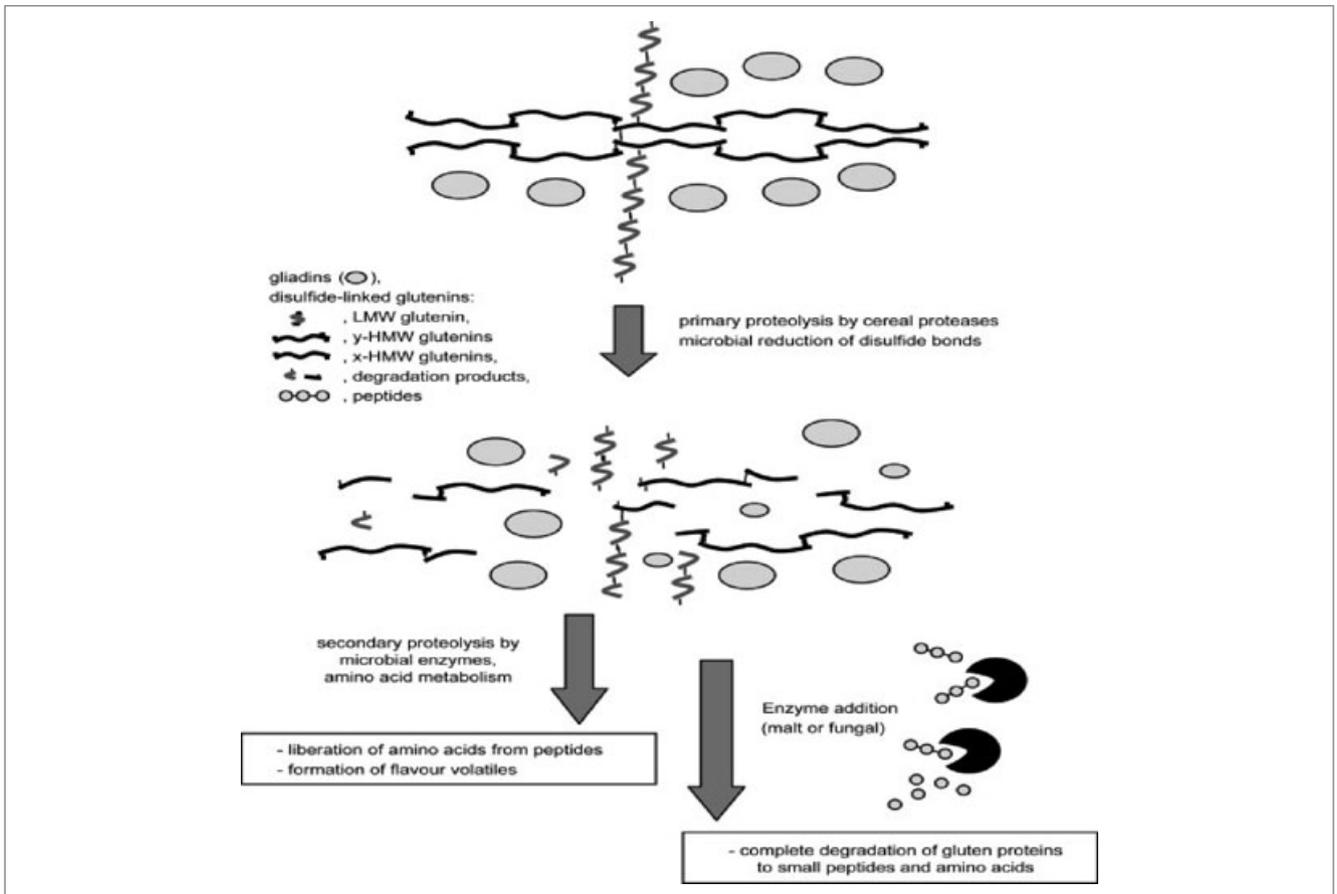


Figure 5–Proteolytic events during sourdough fermentation: key enzymes and metabolic activities for primary and secondary proteolysis. The representation of the gluten macropolymer is based on Wieser (2007).

Table 3–Volatile compounds identified in imitated and inoculated wheat sourdoughs.

	Alcohols	Esters	Carbonyls and others
Imitated sourdough ^a	Propanol, pentanol	Ethyl acetate (tr)	Hexanal, benzaldehyde(<i>E</i>)-2-heptenal
Sourdoughs fermented with starter cultures	Ethanol, methylpropanol, butanol, pentanol, hexanol, 2-hexanol, (<i>E</i>)-2-hexenol, heptanol	Ethyl acetate, ethyl lactate, ethyl octanoate, hexyl acetate	Hexanal, (<i>E</i>)-2-heptenal, 2-pentylfuran
Sourdoughs fermented with starter cultures and sourdough yeasts	Ethanol, propanol, methylpropanol, 2-butanol (tr), butanol, 2- and 3-methylbutanol, pentanol, hexanol, 2-hexanol, (<i>E</i>)-2-hexenol, heptanol, octanol (tr)	Ethyl acetate, ethyl propanoate, butyl acetate, 2-methylbutyl acetate, pentyl acetate, ethyl hexanoate, hexyl acetate, ethyl lactate, ethyl octanoate	3-Hydroxy-2-butanone (acetoin), 2,3-butanedione (diacetyl), (<i>E</i>)-2-heptenal, 2-pentylfuran

^aThe imitated sourdough was manufactured by addition of lactic and acetic acid in the same level as in sourdoughs and kept under the same conditions (Hansen and Hansen 1996).

heterofermentative bacteria (Gobbetti and others 1995b) that acidify, decrease pH, and contribute aroma to the bread dough (Galal and others 1978; Barber and others 1985). The 2nd category is volatile compounds that include alcohols, aldehydes, ketones, esters, and sulfur. All these compounds are produced by biological and biochemical actions during fermentation (Spicher 1983). The generation of sufficient amounts of volatile compounds during fermentation needs a multiple-step process of about 12 to 24 h, while fermentation by baker's yeast alone is finished within a few hours. The generation of volatiles in sourdoughs is clearly influenced by the activity of the LAB and the sourdough yeasts. Factors influencing their activity, such as temperature and water content, will consequently influence the amounts of the metabolites formed. Generally, LAB are mostly responsible for acidification. The key degradation reaction of amino acids during dough fermentation is the Ehrlich pathway leading to aldehydes or the corresponding alcohols, respectively (Schieberle 1996). Contrary, during baking, the *Strecker* reaction, initiated by α -dicarbonyl compounds such as methylglyoxal (2-oxopropanal), also leads to the respective aldehydes, but also to the corresponding acids (Hofmann and Schieberle 2000). Because the chemical structures of the aldehydes generated during the Ehrlich pathway and the *Strecker* reaction are identical, it is a challenge to quantitatively differentiate between the amounts generated from the respective pathways (Table 3). During dough fermentation, also phenylpropanoic acids, such as ferulic acid, were shown to be significantly increased (Hansen and others 2002).

Beneficial Applications of Sourdough Technology

Dietary fiber (DF) in sourdough

There is growing consumer interest in health aspects of food, including functional food products with specific physiological functions of health relevance. However, good sensory properties remain a prerequisite for any successful food, and consumers also expect food to fulfill other criteria such as safety and convenience. Nutritionally, cereal foods are an important source of carbohydrates, protein, DF, and many vitamins and nonnutrients. DF has long been considered the major health-protective component of grains. There is now increasing evidence also of other protective compounds, such as oligosaccharides and phytochemicals, which together with DF are concentrated in the outer layers of the grains. The levels and also bioavailability of carbohydrates and various bioactive compounds can remarkably be influenced by processing. Another example of the potential of sourdough is the ability to modify the bran fraction of the grain (rich in fiber) in such a way that larger amounts of bran can be utilized in breads. The nutritional importance of DF has been demonstrated in many

studies. A typical Western diet contains less than 20 g/d, whereas the recommended daily intake is 25 to 30 g. Currently, most people eat too little fiber and these low levels of DF in Western diet contribute to a long list of diseases, ranging in severity from dental caries through constipation to obesity, colorectal cancer, coronary heart disease, and type 2 diabetes. The most common source of DF in baking is cereal bran, especially wheat bran. The use of barley bran derived from huskless or dehusked barley or oat bran derived from husked oat kernels is also becoming more widespread due to their high soluble DF content, in particular their content of mixed-linked β -glucan. According to Seibel (1983), addition of fiber causes the following technological changes: (1) increases DH, (2) results in a moister and shorter dough, (3) decreases fermentation tolerance (that is, dough is able to keep the optimum volume a shorter time during proofing), (4) decreases bread volume, (5) creates a crumb that is tense and nonelastic, and (6) creates flavor changes depending on type of fiber and bread type. However, additions of cereal bran, especially in such amounts that health benefits can be expected, cause severe problems in bread quality.

EPS from *Lactobacilli*

The addition of plant polysaccharides is a common practice in the production of bread or frozen dough to improve textural properties and shelf life of bread. Dextran from *Leuc. mesenteroides* finds commercial application in baking improvers (Decock and Cappelle 2005). Tieking and others (2003a, 2003b) reported that the addition of dextran to a level of 5 g/kg flour affected the viscoelastic properties of wheat doughs and the volumes of the corresponding breads to a greater extent than addition of the same levels of reuteran or levan. Polymers produced from *Lactobacilli* thus may be expected to beneficially affect one or more of the following technological properties of dough and bread: (1) water absorption of the dough, (2) dough rheology and machinability, (3) dough stability during frozen storage, (4) loaf volume, and (5) bread staling.

Improving nutritional and sensory quality

LAB have a long history of use in food and are generally regarded as safe organisms (Magnusson and others 2003). Cereal grains, like wheat from that most breads is produced, are low in some of the essential amino acids, such as lysine, threonine, methionine, tryptophan, and isoleucine. Therefore, cereal grains can be considered to be low in high-quality protein. Thus, cereal grain-based diets, prevalent in many areas of the world, may be deficient in some essential amino acids. Lyophilized cultures of microorganisms may be added to cereal grains, such as wheat, in bulk to increase their basic nutritive protein quality (El-Megeed

and others 1989). The mineral availability of sourdough baked goods is then also improved (Larsson and Sandberg 1991; Lopez and others 2003). The low pH values associated with chemically or microbiologically acidified wheat dough lead to solubilization of the phytate complex, thus increasing mineral bioavailability. EPS produced by *L. sanfranciscensis* improve the nutritional properties of sourdough fermented products in view of the fact that they may be metabolized by bifidobacteria (Korakli and others 2001). Some sourdough LAB have shown hydrolyzing activities toward prolamin peptides involved in human cereal intolerance (Di Cagno and others 2002).

Flavor development

The flavor of leavened baked goods is influenced by raw materials, by sourdough fermentation, by the type of starters, and by proofing and baking conditions. The ratio between lactic acid and acetic acid is an important factor affecting the aroma of the final bread (Corsetti and Settanni 2007), and it is influenced by the fermenting microorganism, the fermentation temperature, and the type of flour (Hansen and Schieberle 2005). The optimal use of sourdough can improve the taste and flavor of the bread (Rehman and others 2006). The flavor of sourdough wheat bread is richer and more aromatic than wheat bread, a factor that can be attributed to the long fermentation time of sourdough (Brummer and Lorenz 1991). The concentration of 2-phenylethanol, one of the most potent odorants of wheat breadcrumb increases in sourdough breadcrumb (Gassenmeier and Schieberle 1995). Recently, fermentation was shown to induce production of flavoring compounds also in gluten-free products. For example, fermentation of sorghum for production of towga generated different flavoring compounds (Mugula and others 2003). Alcohols were produced in high concentration when fermentation was carried out with *Lb. orientalis* in combination with *L. brevis* or *Lb. plantarum*, and diacetyl was produced in significant amounts by *Lb. plantarum* and *Pediococcus pentosaceus*. The production of aldehydes was increased by cofermentation with *Lb. plantarum* and yeasts. Fermentation with mixed cultures containing *Lb. plantarum* enhanced the diacetyl content in maize fermented meals (Edema and Sanni 2008). In addition to the polymer formation in dough, EPS-forming *Lactobacilli* for use in baking applications should also exhibit additional metabolic traits to achieve improved flavor, texture, and shelf life of bread. For example, EPS-forming strains of the species *L. pontis* and *Lb. reuteri* also exhibit arginine metabolism with positive effects on bread flavor. Moreover, EPS-forming strains of *Lb. reuteri* that additionally produce reutericyclin in dough to delay growth of rope-forming bacilli in bread are available (Gänzle and Vogel 2002; Thiele and others 2002; Tiekling and others 2003c).

Dough structure and bread characteristics

The sourdough fermentation affects dough rheology at 2 levels, in sourdough itself, and in bread dough-containing sourdough. In dough, fermentation decreases elasticity and viscosity, whereas the addition of sourdough to final bread dough results in less elastic and softer dough (Clarke and others 2004). Di Cagno and others (2002) measured the rheology of fermented dough by using empirical techniques and found a decrease in resistance to extension and an increase in both extensibility and degree of softening. During the sourdough fermentation, different organic acids are produced. These organic acids improve the flavor of bread, help the swelling of gluten, and increase gas retention, resulting in products with good texture and massive volume and also functioning as natural

dough conditioners (Park and others 2006). EPS produced by LAB during fermentation is one of the aspects of sourdough technology with the potential for the replacement by hydrocolloids (Korakli and others 2001).

Acids produced during fermentation strongly influence the mixing behavior of dough, and dough with lower pH value requires a slightly shorter mixing time (Hoseney 1994). The pH of a ripe sourdough varies with the nature of the process and starter culture used, but for wheat sourdoughs it ranges from 3.5 to 4.3. The nature of the flour, in particular its ash content, has a considerable effect on acidification (Collar and others 1994; Clarke and others 2002). The acid increases the solubility of the glutenin fraction extracted from wheat flour and also affects the swelling power of gluten (Axford and others 1979). In comparison to bread prepared with baker's yeast, the sourdough breads are characterized by moist, dense grains, and a rather chewy texture (Qarooni 1996). The application of sourdough to wheat breads has a positive impact on bread volume, which is a primary quality characteristic of bread (Collar and others 1994; Clarke and others 2004). Holes of relatively small size (1 or 2 mm) are required in bakery products, whereas large voids or irregular crumb distributions are undesirable (Cauvain 1998). An increase in the mean cell area has been demonstrated via addition of 20% sourdough that increases the acceptability of the product (Crowley and others 2002).

Shelf life

In bakery products, staling indicates decreasing consumer acceptance that is caused by changes in crumb other than those resulting from the action of spoilage organisms (Bechtel and others 1953). Contamination of bread occurs after baking, and airborne distribution of dust and mold spores is the main cause for bread spoilage (Legan 1993). In addition to economical losses, bread spoilage also represents a health hazard for consumers, especially when bread is contaminated with mycotoxigenic molds. The application of LAB in the form of sourdough has a positive effect on bread staling. One such effect is an improvement in loaf-specific volume, which is associated with the reduction in the rate of staling (Axford and others 1968). The breads containing sourdough can decrease the staling rate as measured by differential scanning calorimetry (Corsetti and others 2001). Sourdough-associated LAB produce many antimicrobial substances, such as organic acids, CO₂, ethanol, hydrogen peroxide, diacetyl, fatty acids, phenyllactic acid, reuterin, and fungicins (Messens and De Vuyst 2002; Schnürer and Magnusson 2005). Among the organic acids, acetic and propionic acid produced by heterofermentative LAB are more effective than lactic acid (Schnürer and Magnusson 2005). Caproic acid produced by *L. 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 and others 1996). Also, *L. plantarum* shows very broad antimicrobial activity, and the antifungal compounds 4-hydroxyphenyllactic and especially phenyllactic acids have been identified as responsible for fungal inhibition (Dal Bello and others 2007; Lavermicocca and others 2000; Ryan and others 2009). A synergistic effect was found when sourdough fermented with antifungal *L. plantarum* strains was used in combination with calcium propionate for production of wheat bread (Ryan and others 2008). In sourdough, *L. reuteri* has shown to produce, in active concentrations reutericyclin, a LMW antibiotic acting against Gram-positive LAB and yeasts (Gänzle and others 2000). *Lactobacillus reuteri* strains have also been shown to produce reuterin, an antimicrobial substance active against bacteria,

yeasts, and fungi (Gänzle 2004). Sourdough-associated LAB are also effective against rope spoilage of bread induced by *Bacillus* spp., probably due to production of organic acids and other still unknown antibacterial substances (Katina and others 2002; Valerio and others 2008).

Antibacterial and antimold activity

In general, LAB play a crucial role in the preservation and microbial safety of fermented food, thus promoting the microbial stability of the final products of fermentation.

Antibacterial activity

Bread and other leavened baked products can become contaminated with spoilage bacteria or molds. LAB have been shown to possess both antibacterial and antifungal properties and sourdough addition is an effective procedure to preserve bread from spoilage, since it complies with the consumer request for additive-free products (Messens and De Vuyst 2002). Besides adding various compounds (for example, organic acids, hydrogen peroxide, diacetyl), sourdough LAB can inhibit the growth of other, usually related microorganisms by producing bacteriocins or other substances, such as the low-molecular-mass antibiotic reutericyclin produced by *Lb. reuteri* LTH2584 (Höltzel and others 2000). Antibacterial compounds may also regulate microbial interactions in complex food systems. Only a few bacteriocins or bacteriocin-like inhibitory substances (BLIS) produced by sourdough LAB have been characterized. They include bavaricin A by *L. bavaricus* MI401 (Larsen and others 1993), plantaricin ST31 by *Lb. plantarum* ST31 (Todorov and others 1999), and BLIS C57 by *Lb. sanfranciscensis* C57 (Corsetti and others 1996). LAB bacteriocins have shown a capability of inhibiting foodborne pathogens and/or food spoilage bacteria, including *Listeria monocytogenes*, *Bacillus subtilis*, and *Staphylococcus aureus*; thus, their use as food additives or the application of the producer strains as starter or protective culture might contribute to the manufacturing of safer products. Furthermore, bacteriocins may lead to a reduction of added chemical preservatives used by the food industry (Messens and De Vuyst 2002).

Antimold activity

The most frequent cause of spoilage in baked goods is represented by fungal growth, which may cause public health concerns if mycotoxin production is involved (Legan 1993). Spoilage fungi commonly associated with bakery losses belong to the genera *Aspergillus*, *Cladosporium*, *Endomyces*, *Fusarium*, *Monilia*, *Mucor*, *Penicillium*, and *Rhizopus* (Legan 1993; Keshri and others 2002). A mixture of acetic, caproic, formic, propionic, butyric, and *n*-valeric acids, acting in a synergistic way, in which caproic acid plays a key role, was found to be responsible for the *in vitro* inhibitory activity of *Lb. sanfranciscensis* CB1 against molds responsible for bread spoilage by *Fusarium*, *Penicillium*, *Aspergillus*, and *Monilia* (Corsetti and others 1996). Lavermicocca and others (2000) purified and characterized 2 antifungal compounds produced by *Lb. plantarum* ITM21B, identified as phenyllactic and 4-hydroxy-phenyllactic acids, which still retained their fungicidal activities after baking. *Lactobacilli* able to produce the above 2 antifungal compounds, among which phenyllactic acid is the most potent one, were shown to delay *Aspergillus niger* and *Penicillium roqueforti* growth for up to 7 d and significantly prolong the shelf life of bread. It has been found that fungicins are produced by strains of *Lb. casei*, *Lb. pentosus*, *L. paracasei* subsp. *Paracasei*, and *Lb. coryniformis* subsp.

coryniformis (Gourama 1997; Okkers and others 1999; Magnusson and Schnürer 2001).

Conclusion

Sourdough technology, although a traditional process when combined with modern manufacturing techniques, could yield healthier products for consumers. Sourdough helps to facilitate desirable texture and loaf volume to breads. Moreover, sourdough is also effective in the production of EPS and manufactures products with a high level of DF and enhanced palatability. Sourdough has also been shown to be useful in the production of breads with slow starch digestibility and hence low glycemic responses. 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 use of sourdough is useful for making bread products with an increased level of flavor compounds, ultimately increasing the evenness in the batches and customer satisfaction. Sourdough technology can also be useful to reduce or eliminate the level of preservatives often used in baked products, as sourdough has shown antibacterial and antimold activity. Use of sourdough can also be extended to other products such as biscuits, pizza, snack foods and also products with multigrain flour or with enriched DF. Thus, sourdough could be useful in serving mankind with a wholesome, tasty, and convenient foods.

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