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REVIEW ARTICLE

Functional and Technological Aspects of Sourdough Fermentation with *Lactobacillus sanfranciscensis*

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Abstract In recent years, there has been a growing interest in sourdough bread, which is a traditional product with its lasting production since ancient times. Studies related to the characterizations of sourdough lactic acid bacteria, their contribution to consumer health, dough structure, bread texture and shelf-life have been carried out. In this review, one of the key lactic acid bacteria of sourdough, *Lactobacillus sanfranciscensis*, is discussed from several aspects. The importance of San Francisco bread for consumer health is also emphasized. In addition to the contribution of *L. sanfranciscensis* to the textural properties and shelf-life of sourdough bread; the health benefits, flavour and antimicrobial substances, rheological characteristics of sourdough fermented by *L. sanfranciscensis* are reviewed. The functional aspects of sourdough fermented by *L. sanfranciscensis* are also demonstrated such as offering alternative bakery products for coeliac patients, diabetics or consumers having allergy to the bakery products fermented with baker's yeast (*Saccharomyces cerevisiae*) that are known as anti-*S. cerevisiae* antibodies.

Keywords Sourdough fermentation · *Lactobacillus sanfranciscensis* · San Francisco bread · Bakery products

Introduction

Lactic acid bacteria have a long history of use in a wide variety of cereal fermentations, especially in the manufacture of baked goods [18]. In addition to yeast fermentation, the action of lactobacilli in the dough has a significant effect on the generation of flavour in bread [110]. Sourdoughs, which are dominated by a complex microflora composed of yeasts and lactic acid bacteria, result in breads having improved crumb structure [89], volume, texture and sensory properties [82], and nutritional value as well as having enhanced shelf-life properties [18] compared to straight doughs. Lactic acid bacteria (LAB) are numerically and metabolically the predominant organisms in wheat and rye sourdoughs [41].

Sourdough can be defined as "a dough whose microorganisms originate from mother sponge or its starters and are metabolically active or can be reactivated, upon addition of flour and water they continue to produce acid" [6]. Sourdough is widely used in the biotechnology of baked goods because of the many advantages it offers with respect to the baker's yeast, which is classed as *Saccharomyces cerevisiae* and is the primary fermentative agent in bread production [25, 110]. These advantages are mainly related to the metabolic activities of sourdough LAB such as lactic acid fermentation, proteolysis and synthesis of flavour compounds and prevention of microbial contamination [25].

Sourdough was used as a leavening agent in bread production until it was replaced by baker's yeast in the nineteenth century; from then on its use was reduced to artisan and rye bread. In modern baking, leavening of bread dough is accomplished by the addition of baker's yeast into bread dough. Even though sourdough process is challenging technologically since the longer fermentation process prevents high output in bread production, today an increasing number of consumers are interested in bread made without baker's yeast. Nowadays, sourdough is employed in the manufacture of a variety of products such as breads (i.e. San Francisco bread, Rewena bread,

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Ruisleipä, Carasau, Zichi, etc.), cakes (i.e. Pandoro, Colomba, Panettone, Cornetto Brioche, etc.) and crackers, whilst being applied to a large variety of cereal flours throughout the world. The use of sourdough to manufacture cakes is mostly common in Italian bakery for traditional products. Foschino et al. [34] mentioned about the predominance of *L. sanfranciscensis* in sourdoughs used to prepare many baked products of the Italian food tradition such as *Brioches* for breakfast, *Panettone* and *Pandoro cakes*. Gobbetti and Corsetti [45] and Stolz [97] reported that the association between *L. sanfranciscensis* and *Scinax exiguus* was typical for the production of *Panettone*. Picozzi et al. [84] reemphasized that there was a mutualistic relationship, based on non-competition of carbon source, between *L. sanfranciscensis* and a maltose-negative yeast for many Italian sweet bakery products such as *Panettone, Pandoro, Colomba, Cornetto Brioche* and similar snacks. Vernocchi et al. [106] characterized the predominant microorganisms involved in the production of *Colomba* as *C. milleri* and heterofermentative LAB (such as *L. sanfranciscensis*). De Vuyst and Neysens [22] stated that sweet-leavened baked products obtained from sourdoughs were typical in northern Italy and were traditionally made for religious festivities. *Panettone cake* in Milan and *Pandoro* in Verona are manufactured for Christmas, whilst *Colomba* is a Milanese cake for Easter. Moreover, snacks for breakfast or coffee time, such as *Cornetto, Pandorino and Brioche*, and other small cakes for infants are typical Italian bakery products. See the study of Garofalo et al. [37] for *Panettone* production, the study of Vernocchi et al. [106] for *Colomba* production, the study of Pagani et al. [80] for *Panettone, Pandoro and Colomba cake* production processes. Wheat sourdough is used in more than 30 % of Italian bakery products, including more than 200 different types of sourdough breads, whilst bread leavened by endogenous sourdough yeast instead of baker's yeast is still occasionally used in industrial scale rye bread production in Finland. The Mediterranean area specializes in sourdough wheat products, whereas in Central Europe and Scandinavia, bread made from rye, wheat or a mixture of these flours is a staple in the diet. In Poland, 70 % of the bread is baked from rye sourdough (mixed rye-wheat bread). The cereal intake in the traditional diet of Greece is mostly in the form of sourdough bread rather than pasta. In Morocco, most people eat home-made bread made with traditional sourdough, which has been carefully kept in every family. Addition of baker's yeast is used mainly in towns and villages where refrigeration can be employed. San Francisco sourdough bread is another example for the products that is produced by the application of endogenous leavening [13, 29, 56, 57].

In this review, *L. sanfranciscensis,* which is known as one of the key organisms in sourdoughs, its role in sourdough fermentations and the interactions of it between yeasts, were reviewed in detail, whilst the contribution of lactobacilli, especially *L. sanfranciscensis,* to flavour, texture, shelf-life and nutritive properties of sourdough breads are emphasized.

Types of Sourdough

Sourdoughs, on the basis of the technology applied, have been grouped into 3 types: type I, type II and type III [13, 23]. This classification is depicted in Fig. 1.

Type I Sourdoughs that are produced with traditional techniques and are characterized by continuous (daily) propagation to keep the microorganisms in an active state, as indicated by high metabolic activity, above all with regard to leavening, that is, gas production [74]. Generally, three-stage fermentation processes are used at a temperature below 30 °C [35].

Type II An industrial type of sourdough used adapted strains to start fermentation [23]. These type of sourdoughs, often used as dough-souring supplements during bread preparation, are semi-fluid silo preparations characterized by long fermentation periods (from 2 up to 5 days) and higher fermentation temperatures (>30 °C) to speed up the process [13]. The industrialization of the baking process for rye bread, and the industrial demand for faster, more efficient, controllable and large-scale fermentation processes resulted in the development of type II sourdoughs [22]. Type II sourdoughs are easy pumpable in an industrial bakery due to their semi-liquid characteristic [23]. In type II doughs, leavening is sustained by the addition of baker's yeast, whereas the traditional type I doughs are dominated by heterofermentative LAB, such as *L. sanfranciscensis* and *L. pontis.* Type II sourdoughs contain homofermentative species, such as *L. amylovorus* and *L. delbrueckii*, in addition to baker's yeast [89].

Type III Sourdoughs are dried doughs containing LAB resistant to the drying process, which are used as acidifier supplements and aroma carriers [13, 74]. Type III processes are initiated by defined starter cultures [89, 107]. As well as type II, types III doughs require the addition of baker's yeast for leavening [74]. The type III sourdoughs are the most convenient way to introduce authentic bread taste into the current high-tech bakery industry [23].

Lactobacillus sanfranciscensis is considered a key microorganism, being the predominant bacterial species in type I sourdoughs for San Francisco bread and many traditional Italian and German bakery products [85].

San Francisco bread is a traditional bread in the Californian region. In the San Francisco Bay area, sourdough

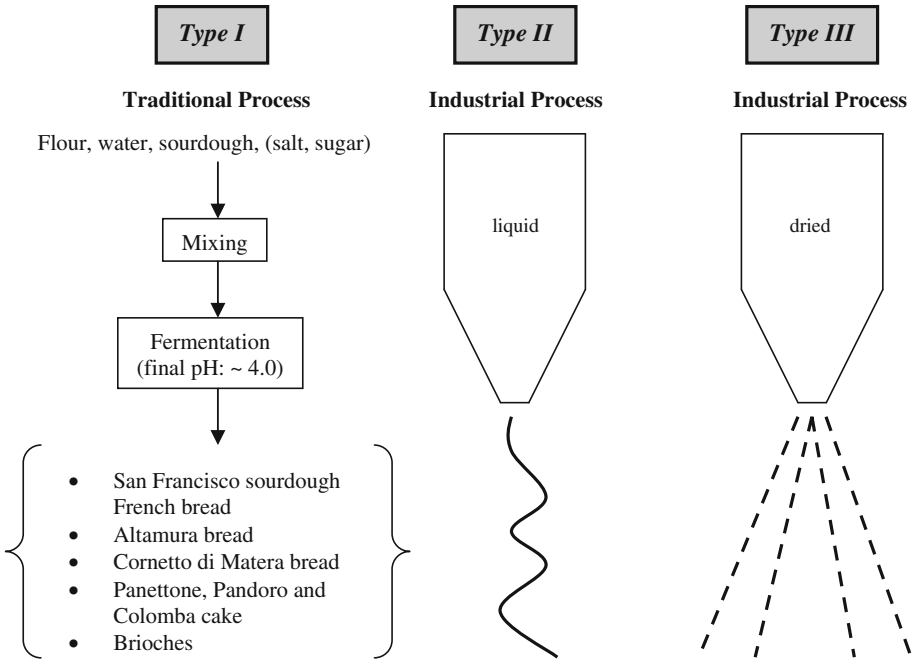


Fig. 1 Types of sourdough

consumption has constituted more than 20 % of all bread produced in the area. It is believed that the strains were imported by French gold diggers and maintained since then. A very typical microflora is present in the real San Francisco sourdough. *Lactobacillus sanfranciscensis* is the predominant lactic acid bacterium in French sourdough bread [23, 99]. It has also been widely isolated from rye and wheat sourdoughs of several bread-producing areas [45].

A major portion of the sourdough bread in the San Francisco Bay area has been produced by using the natural "mother sponge" or sourdough starter. The procedure of preparing the sourdough sponge (mother sponge) and the bread dough is given in Table 1. Approximately 100 parts of the previous sponge is mixed with 100 parts of high-gluten flour and 46–52 parts of water. The way to produce traditional San Francisco French bread is to ferment the sourdough at very low temperatures during a long time. Thus, a sharp acetic acid flavour develops. The starter begins with a pH of 4.4 and levels off at pH of 3.9, an extremely acidic system. It takes about 8 h for the starter to fully develop. When the final dough is made, up to 20 % of sourdough is used on flour weight, with 60 % of water and 2 % of salt, and fermented for a second time at low temperature (4 °C). No sugars, shortening, non-fat milk solids, yeast foods, oxidizing agents, dough softeners or conditioners are used. After about 1 h floor time, the bread dough is moulded and proofed for about 8 h. This time the pH of the bread dough drops from about 5.3–3.9. After the formation of small blisters on the surface is observed, it is baked for a relatively long time (45–50 min) at a relatively low temperature (190–200 °C) with low-pressure steam introduced during the first half of the baking cycle or until the crust begins to develop colour. The crispy reddish crust of the bread shows the typical fish eye. The production process of one of the typical examples of sourdough type I, San Francisco bread, is demonstrated in Fig. 2. It was reported that a pasteurized San Francisco sourdough is now available on the market in liquid form. This liquid sourdough may be dosed automatically in the bakery and may be made less than 3 h having most of the typical properties of the traditional product by the addition of some more yeast into the formulation [23, 99].

Type I sourdoughs are produced with traditional techniques and are characterized by continuous, daily refreshments to keep the microorganisms in an active state [13]. De Vuyst and Neysens [22] classified Type I sourdoughs into 3 groups: (1) type Ia: pure culture sourdoughs that are derived from natural sourdough fermentations (e. g. *L. sanfranciscensis* + *S. exiguus* for the production of San Francisco French bread); (2) type I b: sourdoughs consist of obligate heterofermentative strains of *L. sanfranciscensis,* selected only by the environmental conditions induced by the sourdough fermentation technology applied (e. g. *L. sanfranciscensis/L. pontis* + *C. humilis*); (3) type Ic: sourdoughs such as African sorghum sourdoughs that are produced at higher temperatures (>35 °C), (e. g. *L. fermentum, L. pontis, L. reuteri, L. amylovorus* + *Issatchenkia orientalis*).

Table 1 Formulations for the San Francisco sourdough bread

Starter sponge formulation*	Bread dough formulation*
Flour: 100 (high-gluten four)	100 (regular patent)
Sponge: 100 (previous-40 % of final mix)	20 (11 % of final mix)
Water: 46–52	60
Salt: –	2

*The amounts of the ingredients are given as % flour weight

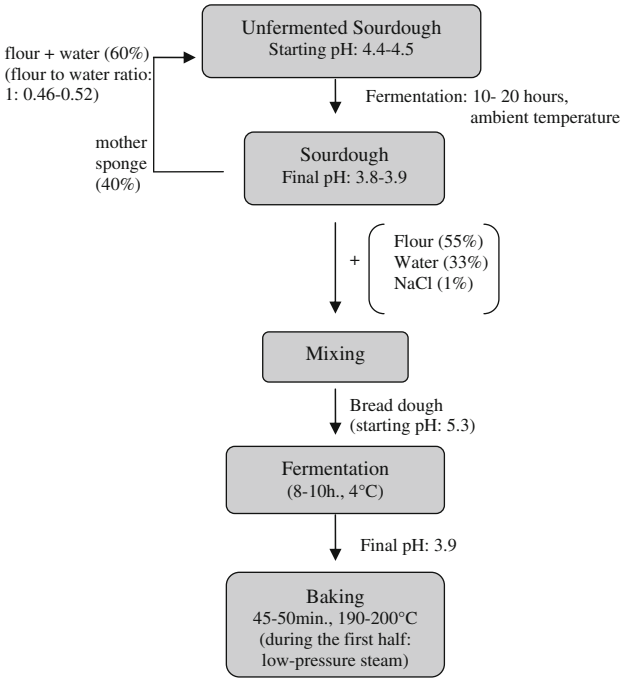


Fig. 2 Production process of San Francisco French bread

Unlike type I sourdoughs, doughs belonging to type II and III require the addition of baker's yeast (*Saccharomyces cerevisiae*) as a leavening agent [13].

Microflora of Sourdough

A number of species of LAB and yeast are responsible for dough transformation. These microorganisms are usually contaminants originating from flour or the environment. Several yeast species are found in sourdoughs, the most prevalent being *Saccharomyces cerevisiae, Saccharomyces exiguus, Candida milleri, Pichia norvegensis, Hansenula anomala* and *Candida krusei* [6, 52]. On the other hand, sourdough lactic acid bacteria generally are from the genera *Lactobacillus, Leuconostoc, Pediococcus* or *Weissella* and the majority of strains belongs to the genus *Lactobacillus* [40]. Although a large variety of LAB has been isolated from sourdoughs, only a few *Lactobacillus* species are highly adapted to the sourdough environment and usually dominate industrial and artisan fermentations.

The species *L. sanfranciscensis, Lactobacillus plantarum, Lactobacillus pontis, Lactobacillus rossiae* are recognized as key organisms in sourdoughs [41]. On the other hand, it has been found that the association between the yeast species *S. cerevisiae* and the LAB species *L. sanfranciscensis, Lactobacillus brevis* and/or *Lactobacillus plantarum* was the most common [82]. *L. sanfranciscensis,* an autochthonous species exclusively isolated from sourdoughs, is generally considered the most important lactic acid bacterium in the fermentation of rye and wheat sourdoughs [18]. In recent studies, *L. reuteri* [38], *L. sanfranciscensis* [6, 10, 18], *L. plantarum* [6, 73, 103], *L. pentosus, L. brevis* [6], *L. alimentarius* [73], *L. rossiae* [12, 27], *L. siliginis* [3], *L. nantensis* [33, 102] were isolated from sourdough samples. In Table 2, the predominant LAB and yeasts belonging to traditional sourdough bakery products are depicted. So far, nearly 50 different species of lactic acid bacteria isolated from sourdough have been reported, some of which may be of intestinal origin and due to cross-contamination [13, 22].

Interactions Between *L. sanfranciscensis* and Yeasts

Lactobacillus sanfranciscensis, named after the city from where the sourdough microorganism was first isolated, is an obligatory heterofermentative *Lactobacillus* with phylogenetic relationship to the *Lactobacillus casei–Pediococcus* group. It was first isolated by Kline and Sugihara [60] and subsequently revised by Weiss and Schillinger [109] for inclusion in Approved List of Bacterial Names. DNA–DNA hybridization showed that *L. sanfranciscensis* is synonymous with *Lactobacillus brevis subsp. lindneri* [45]. It was then isolated from many traditional bakery products, and some of the typical examples for these bakery products have been listed in Table 3.

Lactobacillus sanfranciscensis is an obligate heterofermentative lactic acid bacteria species, which means that it ferments hexoses (monosaccharides with six carbon atoms such as glucose, fructose, galactose etc.) to lactic acid, acetic acid (ethanol), CO_2 and ferments pentoses (monosaccharides with five carbon atoms such as arabynose, xylose, etc.) to lactic and acetic acid. It can produce large amounts of lactic acid and acetic acid from maltose, which is a disaccharide formed from two units of glucose joined with α (1, 4) bond [22, 56]. It requires fresh yeast extractives, unsaturated fatty acids (mainly oleic acid), and it preferentially ferments maltose rather than glucose. The growth of *L. sanfranciscensis* has been found to be related to the availability of specific amino acids and peptides excreted by yeasts [45]. This lactic acid bacterium, which forms a mutualistic association with maltose-negative

Table 2 Sourdough bakery products and their predominant microorganisms

Product	Country	LAB	Yeast	References
San Francisco sourdough French bread	USA	*L. sanfranciscensis*	*S. exiguus* (*T. holmii*)	[60, 98]
Maize bread sourdough	Portugal	*L. brevis, L. curvatus, L. lactis* spp. *lactis, E. durans, E. casseliflavus, E. faecium, S. constellantus, S. equinus*	*S. cerevisiae, C. pelliculosa*	Rocha and Malcata [93]
Rye sourdough	Sweden	*L. fermentum*	–	[21]
Rye sourdough	Russia	*L. plantarum, L. brevis,*	–	[21]
Wheat sourdough	Greece	*L. sanfranciscensis, L. brevis, L. paralimentarius, W. cibaria, L. fermentum*	–	[21]
Rye bread (Reinzuhtsauer)	Germany	*L. sanfranciscensis, L. brevis*	–	[71]
Rye sourdough	Germany			[74]
Type I		*L. sanfranciscensis, L. mindensis*		
Type II		*L. crispatus, L. pontis, L. panis, L. frumenti*	–	
Puglieser bread	Italy	*L. plantarum, L. brevis, L. fermentum, L. fructivorans*	*S. cerevisiae*	[79, 97]
Panettone	Italy	*L. sanfranciscensis*	*C. holmii*	[79, 97]
Colomba cake	Italy	*L. sanfranciscensis*	*C. milleri*	[106]
		S. cerevisiae		[84]
Moddizzosu bread	Italy	*L. pentosus, L. sakei, L. alimentarius*	*S. cerevisiae* (for daily production)	[6]
Sourdough	Belgium	*L. paralimentarius, L. pontis, L. sanfranciscensis, L. plantarum*	–	[95]
Cornetto di Matera bread	Italy	*L. curvatus, L. plantarum, L. paraplantarum, L. pentosus, Lc.mesenteroides, W. cibaria*	–	[114]
Altamura bread	Italy	*L. plantarum, L. casei, L. paracasei*	*S. cerevisiae*	[7]
Wheat sourdough	Italy			[66]
Type I(B)		*L. brevis, L. plantarum*	*C. humilis, C. milleri*	
Type III (L)		*W. cibaria, L. plantarum, P. pentosaceus*	*S. cerevisiae*	
Steamed bun	China	*L. plantarum, L. casei*	*C. tropicalis, P. stipitis, C. parapsilosis, S. cerevisiae*	[70]
Wheat sourdough	France	*L. plantarum, P. pentosaceus, Lc. Mesenteroides, Lc. citreum*		[92]

LAB: *L: Lactobacillus, Lc: Leuconostoc, W: Weissella, P: Pediococcus, S: Streptococcus, E: Enterococcus; Yeasts: S: Saccharomycess, C: Candida, P: Pichia, T: Torulopsis*

yeasts, influences the organoleptic and structural properties of the final products due to acidification and the liberation of precursors of volatile compounds [18, 34].

Metabolic Activities of *L. sanfranciscensis*

Carbohydrate Metabolism

The lactic acid bacteria: yeast ratio in sourdoughs is generally 100:1 [22]. The association between *L. sanfranciscensis* and *Saccharomyces exiguus* is typical in the production of San Francisco French bread and Panettone [45]. *L. sanfranciscensis* is responsible for the souring activity in French sourdough bread, and it helps in dough leavening by gas production, whilst *S. exiguus* is mainly responsible for the leavening function in this particular acidic environment [22]. *L. sanfranciscensis* hydrolyses maltose by a maltose phosphorylase, which produces a molecule of glucose-1-phosphate that is metabolized further, and a molecule of glucose, which is excreted outside the cell to avoid intracellular accumulation (Fig. 3). Inside the *L. sanfranciscensis* cell cytoplasm, glucose-1-P is converted into glucose-6-P by the action of phosphoglucomutase and then further metabolized through the

Table 3 Sources of isolation of *L. sanfranciscensis*

Origin	Source of isolation	References
California (USA)	San Francisco bread	[60]
Poland	Bread starters	[13, 111]
Italy	*Panettone, brioches*, wheat and rye bread sourdough	[36, 45]
Sweden	Commercial sourdough	[69]
Germany	Rye sourdough sponge	[78]
Morocco	Wheat sourdough	[31]
Denmark	Rye sourdoughs	[55]
Italy	*Pugliese* bread, wheat bread, *brioches, Pandoro* cake, *Panettone* cake	[112]
Italy	Sourdoughs of *San Francisco* bread, rye/wheat bread, *Panettone* cake, *brioches*, *Pugliese* bread, *Pandoro*	[34]
Italy	Apulia sourdough cake, crackers	[10]
Germany	Rye sourdough (type I)	[74]
Germany/Italy	Wheat and rye sourdoughs	[11]
Italy	*Carasau* and *Zichi* sourdoughs	[6]
Italy	Abruzzo region sourdoughs	[104]
Italy	Sourdough	[18]
France	Industrial French sourdoughs	[32]

6-phosphogluconate/phosphoketolase pathway. As long as there is plenty of maltose in sourdough, *L. sanfranciscensis* uses such energetically more favourable pathway in which it is releasing glucose. Maltose-negative yeasts such as *S. exiguus* may in turn utilize such excreted glucose. The sourdough yeast does not assimilate maltose, whereas *L. sanfranciscensis* hydrolyses maltose and excretes one of the glucose molecules to be used for the sourdough yeast. The lack of competition for maltose possibly explains the observed stimulation of *S. exiguus* by *L. sanfranciscensis* in San Francisco sourdough. The glucose uptake of the yeast cell can induce an outflow of amino acids, thus the *L. sanfranciscensis* species demonstrate an accelerated growth rate [13, 56].

Saccharomyces exiguus preferentially uses sucrose and glucose and has a high tolerance to the acetic acid produced by geterolactic metabolism (in the pH range 3.8–4.5). Another possibility is also suggested that *S. exiguus* may coexist with the sourdough bacteria because of its resistance to an antibiotic substance produced by the latter. On the other hand, *S. cerevisiae* mostly consumes maltose and especially glucose. Thus, a decrease in the bacterial metabolism has been observed when associated with *L. sanfranciscensis* [45, 98].

Lactobacillus sanfranciscensis has a positive influence on yeast leavening and CO_2 production [22, 45]. Even though the inoculum amount of the baker's yeast is the major parameter in determining the gas production rates, according to the results of rheofermentometer analyses that Akdogan and Ozilgen [1] carried out, the associative growth of *S. cerevisiae* and *L. sanfranciscensis* decreased at one-third the time necessary to reach the maximum production of CO_2 by the yeast. An increase in the total CO_2 was also observed when associated with *S. exiguus*M14 [45].

Gobbetti [46] reported that the addition of fructose to the sourdough (6 g/kg) produced by *S. cerevisiae* and *L. sanfranciscensis* increased acetic acid production and the LAB cell number, decreased ethanol production, slightly affected CO_2 production and doubled the gas production rate. As a result of the maltose-fructose-mediated co-fermentation, the addition of fructose to the wheat flour induces the utilization of fructose by *L. sanfranciscensis* during fermentation. Compared to the sourdough without fructose addition, a great amount of acetic acid is produced, as well as a lower fermentation quotient, which drops into the optimal range of 1.5–4.0 [45].

Valmorri et al. [104] found out that the association between *L. plantarum* and *L. sanfranciscensis* was observed in most of the sourdoughs samples obtained from Abruzzo region. Gobbetti [46] described such an association for wheat as well as for rye sourdoughs, whilst Corsetti et al. [10] reported the association between *L. plantarum* and *L. sanfranciscensis* was common in Italian sourdoughs.

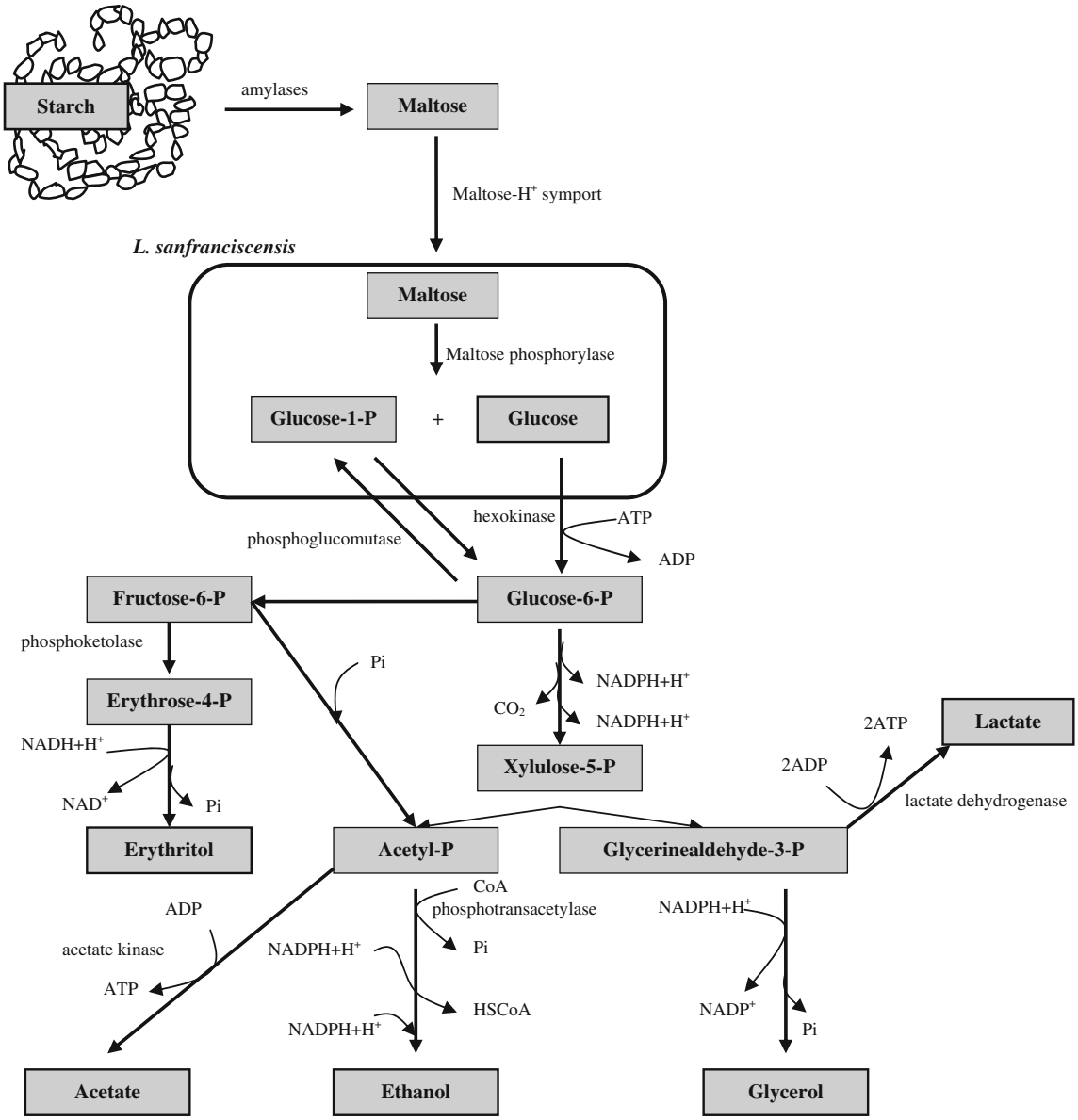


Fig. 3 Utilization of maltose by *L. sanfranciscensis*

Proteolytic Activity of L. sanfranciscensis

The main parameters that influence the kinetics of amino acids in sourdough fermentation are the sourdough microflora requirements, the environmental factors and yeast, which, as a balance between release and consumption, lead to depletion [45].

Yeasts consume amino acids during growth, and the amino acid levels increase only after the yeast growth has ceased. In contrast, in fermentation with defined strains of lactic acid bacteria, the total amino acid levels remain unchanged or are higher when compared to chemically acidified doughs [41]. Vermeulen et al. [105] reported that the amino acid levels in sourdoughs fermented by *L. sanfranciscensis* DSM 20451^T were higher than the doughs acidified chemically. On the other hand, amino acid production by yeasts stimulates *L. sanfranciscensis* growth even though synthetic medium is deficient of essential amino acids such as valine and isoleucine [13]. De Vuyst and Neysens [22] reported that the sourdough yeasts did not affect all the cell yield of *L. sanfranciscensis,* because pH was the limiting factor for growth of the lactobacilli (*e.g. L. sanfranciscensis* does not grow below pH 3.8).

The use of *L. sanfranciscensis* in sourdough fermentation has been related to a considerable increase in the total concentration of free amino acids. When compared to the unstarted sourdough, the sourdough fermented by *L. sanfranciscensis* showed increases in the concentration of

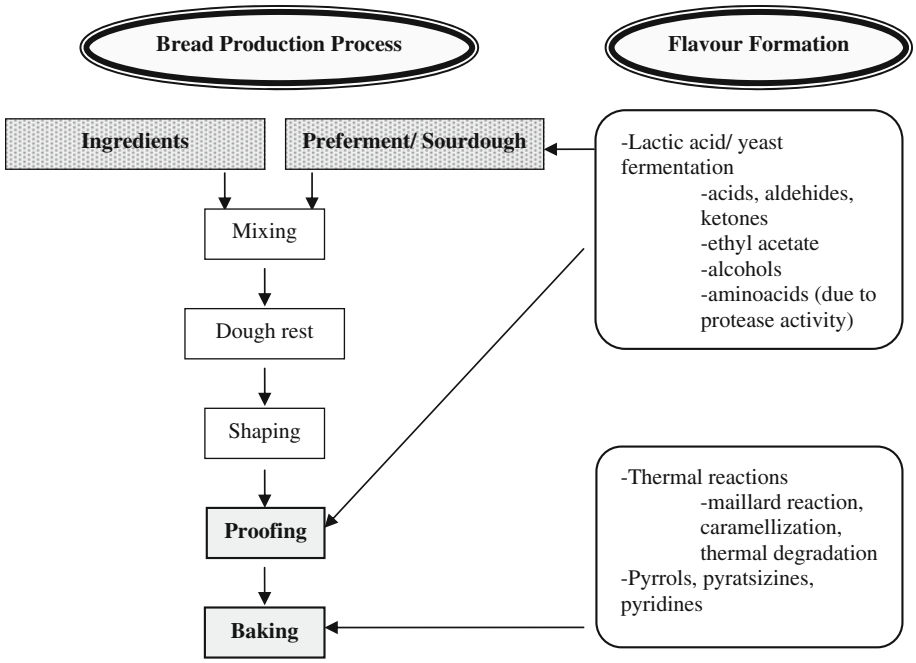


Fig. 4 Flavour formation in sourdough bread during the production process

aliphatic, dicarboxylic and hydroxyl amino acid groups which, for the most part, are stimulatory for the bacterial growth. The peptide hydrolase and proteinase systems of *L. sanfranciscensis* have been characterized and found out that *L. sanfranciscensis* strains showed the highest aminopeptidase, dipeptidase, tripeptidase and iminopeptidase activities compared with the activities of other sourdough LAB [45].

Acidification Rate of L. sanfranciscensis

Gobbetti et al. [43] carried out a characterization of sourdough LAB based on nine parameters of acidification rates. *L. sanfranciscensis* strains isolated from sourdoughs are characterized by the highest variability among the heterofermentative species. They are characterized by a rather long latency phase, high maximum-acidification rate and by tolerance to acidity. These pre-requisites lead to an elevated production of lactic acid (3.48–3.70 g/kg) and acetic acid (0.38–0.44 g/kg).

Vollmar and Meuser [108] reported that the association between *L. sanfranciscensis* and *S. cerevisiae* was optimal for producing acetic acid in continuous sourdough fermentation.

Torulopsis holmii was found to improve dough acidification by *L. sanfranciscensis*, and *S. cerevisiae* enhanced acid production by *L. sanfranciscensis* and *L. plantarum* [46].

Flavour Formation in Sourdough Breads

The flavour of leavened baked goods is influenced by the raw materials [13, 45, 88], sourdough fermentation [13, 56], exogenous enzymes [25], proofing [45], baking [13, 56] and by the type of starters [45, 88]. Figure 4 demonstrates the factors playing role in flavour formation of bread during the production process. Even though the greatest amount of aroma substances is formed during baking, sourdough fermentation is essential for achieving an acceptable flavour, since chemically acidified breads failed in sensory quality [46].

Fermentation determines bread flavour in several ways: (1) microbial conversions of flour components to aroma and aroma precursor compounds and removal of aroma compounds present in the flour; (2) enzymatic conversion of aroma-less precursors present in the flour into aroma compounds [39].

In addition to yeast fermentation, the action of lactobacilli in the dough has a significant effect on the generation of flavour in bread. It has long been recognized that sourdough breads have a much more distinct flavour profile, a higher content of volatile compounds and have higher scores in sensory tests than breads fermented with yeast [56, 110]. In general, the baking process influences the typical aroma of bread crust, whilst dough fermentation is fundamental for the development of crumb flavour [13]. Schieberle [96] identified 14 intense aroma compounds in sourdough bread and attributed the most characteristic aroma of sourdough wheat bread crust to the compound 2-acetylpyrroline [13, 110].

Lactic acid and acetic acid as the basic acids produced by LAB are responsible for the sensory qualities of sourdough bread with citric and malic acids that are also produced by LAB in lesser amounts. The acids are important

for bread flavour, and acetic acid is considered an important enhancer of flavour compounds and together with lactic acid is a catalyst during the Maillard type reaction. The proper ratio between these acids, which is defined as the fermentation quotient (FQ), is necessary for the proper acidity, rich aroma and consequently consumer acceptance with its relevance for the structure of final products. The participation of volatiles in total acidity of bread should not exceed 30 %. Excessive volatile acidity is perceived as astringent and unpleasant. However, low volatile acidity weakens bread aroma [13, 29, 45, 88].

In order to generate sufficient amounts of volatile compounds, the generation process needs multiple steps of fermentation that last about 12–24 h. On the other hand, when baker's yeast is used, the fermentation is completed within a few hours [13]. A study has demonstrated that lactic acid bacteria produced lower concentrations of volatile compounds than yeast in sourdough bread. During sourdough fermentation, high levels of ornithine, leucine, isoleucine, valine and methionine accumulate as a result of microbial or wheat protease activities and amino acid metabolism of lactobacilli. These amino acids are further used by yeast or transformed into aromatic volatiles during fermentation and baking [29, 72].

Along with amino acids, one other key driver of flavour is the carbon source available to the microorganisms. Sucrose addition to wheat dough stimulates both yeast and LAB growth and increases bacterial production of lactic and acetic acids [88]; whilst the addition of fructose as a hydrogen acceptor may increase the production of acetic acid in sourdough fermented with heterofermentative cultures [56].

Lactobacillus sanfranciscensis strains show a wide and homogenous profile of volatiles, which differ greatly from those of the other heterolactic species. It may be defined as unique among the sourdough LAB and irreplaceable in sourdough production. Ethyl acetate, alcohols (ethanol, 1-propanol, 2-methyl-1-pentanol, 1-heptanol and 1-octanol), aldehydes (3-methyl-1-bytanal, heptanal, trans-2-heptanal, octanal and nonanal) and acetic acid are the main compounds produced by *L. sanfranciscensis* [45].

De Angelis et al. [16] demonstrated that the arginine metabolism of *L. sanfranciscensis* had a defined impact on bread flavour. Gänzle and Vogel [38] mentioned about the metabolic activities of sourdough lactobacilli such as the conversion of arginine to ornithine and thus enhancing the roasty flavour of bread.

Meignen et al. [72] reported that fermentation with mixed starters produced significantly ($P < 0.05$) more aroma compounds in sourdough than the single-starter process. They investigated the effects of sourdough fermentation carried out with *L. brevis* and *S. cerevisiae* on the aroma compounds and found out that yeast growth was inhibited by *L. brevis*, but much more acetic acid and aroma compounds were formed with mixed starters (*L. brevis* + *S. cerevisiae*). They also realized that in comparison, bread made from the mixed-starter fermentation process had aroma descriptors such as sugar-, acid- and bitter-type, which were probably related to the high acidity developed by *L. brevis*.

Hansen and Hansen [54] reported that ethanol and ethyl acetate were produced in the highest amounts in sourdoughs fermented by *L. sanfranciscensis*, whilst ethyl-n-propanoate, butyl-acetate and n-pentyl acetate were only produced in sourdoughs started with yeasts [45]. As Meignen et al. [72] mentioned, Gobbetti and Corsetti [45] had also supported the effect of microbial interactions on the volatile synthesis. They reported that sourdoughs started with the association of *L. sanfranciscensis* and other homo- or heterofermentative LAB and/or *S. exiguus* are characterized by a balanced profile. Furthermore, Vollmar and Meuser [108] found out that an association between *L. sanfranciscensis* and *S. cerevisiae* showed optimal production of acetic acid in a continuous sourdough fermentation.

Torulopsis holmii improved dough acidification in association with *L. sanfranciscensis* and *S. cerevisiae* [88]. Further enhanced acid production and guaranteed equilibrated aroma in wheat sourdough breads were observed if associated with *L. sanfranciscensis, L. plantarum* and *S. cerevisiae* [45, 88].

The synergistic metabolic activities of the microorganisms produce an acidification or souring influencing the final character of bread. Therefore, the flavour of sourdough wheat bread is richer and more aromatic than in wheat bread, a factor that can be attributed to the long fermentation time of sourdough [88]. Sensory evaluation of wheat bread crumb showed that bread made with sourdough fermented by the heterofermentative *L. sanfranciscensis* had a pleasantly mild, sour odour and taste. Bread fermented with *L. plantarum* had an unpleasant metallic sour taste, but when the sourdough was also supplemented with the sourdough yeast *S. cerevisiae,* the bread acquired a more aromatic bread flavour. The most desirable sensory characteristics are obtained at pH 4.0–5.5 and at 140 °C. The loaves made with the addition of 5–10 % sourdoughs fermented by *L. plantarum* and 5–10 % sourdoughs fermented by *L. sanfranciscensis* are preferred in odour and taste. Mixed cultures with both LAB and yeast are recommended for an aromatic and pleasant sourdough bread flavour [56, 88].

Nutritional Aspects

Although optimal sensory characteristics represent the basis for any successful fermented food, consumers are

particularly sensitive to its nutritional value and healthy aspects [13]. Food fermented with LAB is a regular element of our diet due to its rich flavour, safety and high nutritive value. Health benefits of sourdough bread are numerous [29]: (1) minerals become bioavailable [24, 39, 56, 81], (2) blood glucose and insulin responses are lowered [29, 56] and (3) a reduction is observed in the levels of gliadin peptides involved in human gluten intolerance [39, 47] via the application of sourdough fermentation and the consumption of sourdough bread.

Reduction in Phytate Content by Sourdough Fermentation

Cereal grains are important sources of minerals such as iron, potassium, magnesium and zinc, but also contain phytic acid, which is the hexaphosphoric ester of the hexahydric cyclic alcohol meso-inositol and is considered to be an antinutritional factor in human diet due to its chelating ability. Phytic acid can form complexes with those minerals due to its highly negative charge and acts as a chelator of these minerals preventing their absorption and thus reducing their bioavailability. Inositol penta- (IP5), tetra- (IP4) and triphosphate (IP3) are also called phytates [13, 65, 81]. The content of phytate is 6 mg/g in rye grain, 3–4 mg/g in flour of soft wheat and 9 mg/g in hard wheat flour [56].

Gänzle and De Vuyst [39] reported that fermentation increased the bioavailability of minerals as a consequence of phytate degradation through microbial and cereal enzymes. Hansen [56] pointed out that sourdough fermentation was shown to be more efficient than yeast fermentation in reducing the phytate content in whole bread (62 and 38 %, respectively).

Phytate degradation in sourdough, resulting from LAB and yeast development, effectively prevents deficiencies of zinc, calcium, iron and other essential minerals [29]. Bread made by sourdough fermentation may result in a more suitable pH condition for the degradation of phytic acid by endogenous phytases and sourdough may also be a source of microbial phytases [17]. Phytase is a generic term to describe an enzyme that hydrolyses phosphomonoester bonds from phytic acid. Phytase [myo-inositol hexakis (dihydrogenphosphate) phosphohydrolase, EC 3.1.3.8] catalyses the hydrolysis of phytic acid into myo-inositol and phosphoric acid via penta- to mono-phosphate [81]. This enzymatic activity produces available phosphate and a non-metal chelator compound. In addition, myo-inositol is evaluated for its ability to improve the mental health of patients with various psychiatric disorders. It is present in human brains [5]. In particular, inositol is active in cell membranes and in sending messages signalling the control of cell functions in the nerve system [19]. Häussinger [58] reported that a first indication of the presence of low-grade cerebral oedema in patients with cirrhosis was derived from H-MRS (Occipital Proton Magnetic Resonance Spectroscopy) studies of the human brain in vivo, and these noted a reduction in the myo-inositol signal in the brain. Furthermore, inositol positively contributes to the metabolism of fats and helps to reduce cholesterol levels in the blood. It clears up cases of slight hypertension by gradually lowering blood pressure and is also useful in treating schizophrenia, hypoglycaemia. Finally, inositol has been found to prevent swelling of the liver [19]. Recent studies also demonstrates that myo-inositol has synergistic or additive effects in inhibiting the development of cancer [5].Thus, phytases are considered to be enzymes of great value in upgrading the nutritional quality of phytate-rich foods as well as their health benefits [17]. Moreover, insoluble protein-phytate complexes are formed at low pH, as found in the stomach of monogastric animals, and protein digestibility is reduced. Dietary phytase supplementation has been shown to prevent the formation of such complexes or to aid in dissolving them faster, thus phytases may improve protein digestibility [13].

Phytase activity is present in grain raw materials, as well as in yeasts and lactic acid bacteria. Phytase activity 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. Leenhardt et al. [67] stated that a moderate decrease of pH to 5.5 during sourdough fermentation was sufficient to reduce phytate content of whole-wheat flour by about 70 % by the endogenous phytase present in the flour [86].

Greiner and Konietzny [50] demonstrated that the phytate content of sourdough rye bread was found to be 0.1–0.3 mg/g (per mass of dry matter), whereas the phytate content of whole rye bread was 1.9–4.3 mg/g. Rizzello et al. [90] pointed out that the sourdough fermentation of wheat germ increased the phytase activity and enhanced the bioavailability of especially Ca^{++}, Fe^{++}, K^{+}, Mn^{++}, Na^{+} and Zn^{++} as a result of the study they carried out about the sourdough fermentation of wheat germ and its nutritional characteristics. They stated that the water-/salt-soluble extract of sourdough fermented wheat germ contained significantly ($P < 0.05$) higher concentrations of free minerals due to the phytase activity than those found in raw wheat germ.

De Angelis et al. [17] investigated the phytase activities of sourdough LAB and reported that the highest values were found for *L. sanfranciscensis* with 420.8 U/ml (after 2 h of incubation at 45 °C). They also demonstrated that the capacity of hydrolysing Na-phytate was largely distributed among sourdough LAB, especially in *L. sanfranciscensis*. On the other hand, Reale et al. [87] investigated phytase production rates of LAB isolated from several

sourdoughs screening 50 different strains and reported that these LAB strains did not reveal significant phytase production [86]. The prolonged fermentation with sourdough enhanced acidification and led to increased solubility of Mg and P. Five different strains of LAB isolated from sourdoughs were tested for their ability to degrade phytic acid, but no difference was observed among strains in the levels of phytic acid hydrolyses [56]. It anyway seems obvious that acid production and therefore lowering of pH are the major mechanisms for LAB to improve mineral bioavailability [86].

Reduction in Glycemic Response with Sourdough Bread

Consumption of sourdough breads reduces the postprandial glucose and insulin responses in humans compared with the consumption of bread without sourdough [39, 68]. After consumption of sourdough bread, satiety is achieved faster, which can be explained by a lower rate of stomach emptying and slower starch digestion [29]. Several mechanisms have been proposed for the ability of sourdough processing to reduce starch digestibility [86]. This nutritional positive effect is assumed to be mainly due to the formation of organic acids, especially lactic acid, during fermentation [39, 56, 86]. Moreover, since the high proportion of non-digestible carbohydrates, such as resistant starch, non-starch polysaccharides and oligosaccharides, was known to contribute to a low glycemic response [49], it is possible to state that exopolysaccharides (EPS) and fructooligosaccharides (FOS) produced by *L. sanfranciscensis* contribute to the same effect [8]. Consequently, sourdough bread meets recommendations for consumption of low GI food, suggesting a protective role against the development of non-insulin-dependent diabetes mellitus and cardiovascular disease [29].

Effect of Sourdough Fermentation on Gluten Intolerance

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 fermentation in baking of gluten-free bread has been efficient in improving product texture and to delay staling of gluten-free breads [86]. Proteolytic activity of lactobacilli [29] and/or proline-peptidases derived from sourdough lactobacilli reduces the levels of gliadin peptides involved in human gluten intolerance, thus may allow new strategies for the production of gluten-free breads [39]. As well as the proteolytic activity of sourdough LAB, the degradation of the cereal proteins in wheat and rye sourdough is related to the acidity. Acidification and the reduction of disulphide 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 (secondary proteolysis). Germinated cereals or other proteases enable an extensive degradation of proteins in sourdoughs during fermentation and may lead to the production of new products for individuals having gluten intolerance [86].

Controlled proteolysis in wheat and rye sourdoughs having extended fermentation time was suggested to reduce gluten levels to an extent that these products are tolerated by coeliac patients [41]. Di Cagno et al. [26] carried out a sourdough fermentation process using flours such as wheat (30 %), oat (10 %), millet (40 %), buckwheat (20 %) and starters such as *L. alimentarius, L. brevis, L. sanfranciscensis* and *L. hilgardii*. They found out that these selected LAB strains almost completely hydrolysed wheat gliadin fractions, whilst prolamins from oats, millet and buckwheat were affected less or not at all after a sourdough fermentation for 24 h- 30 °C. A comparison with a chemically acidified dough or with a dough started with baker's yeast alone showed that the hydrolysis was due to the proteolytic activity of sourdough lactobacilli and that prolamin fractions were not affected during dough fermentation with yeast. As a result of this study, the authors also pointed out that this was the first report of tolerance of coeliac patients for a bread containing 30 % wheat flour on the basis of determination of intestinal permeability during an acute in vivo challenge. Thirteen of the seventeen coeliac patients recruited showed a marked alteration of intestinal permeability after ingestion of baker's yeast bread, whilst the same 13 patients had intestinal permeability values that did not differ significantly from the baseline values when fed the sourdough bread. The other 4 coeliac patients did not respond to the two types of bread.

The FDA is proposing to define "gluten-free" to mean that a food bearing this claim does not contain 20 μg or more gluten per gram of food. The Canadian Food Inspection Agency uses a testing method that has an analytical limitation of 20 parts per million (ppm) gluten, leading to a "gluten-free" label in this country as indicating tested to less than 20 ppm. Similarly, the original 1983 Codex standard, used in much of Europe, defines "gluten-free" foods as consisting of ingredients from wheat, barley, oats, spelt or their cross-bred varieties which have been rendered "gluten-free", with a gluten level not exceeding 200 ppm [14]. Di Cagno et al. [28] screened 46 strains of sourdough LAB containing *L. sanfranciscensis* for proteolytic activity and acidification rate in gluten-free flours in order to investigate their ability to remove gluten and enhance the nutritional properties of gluten-free bread.

They included gluten into formulations before fermentation in order to observe the contamination by gluten. As a result, they found out that the initial gluten concentration of 400 ppm was degraded to below 20 ppm only in the sourdough gluten-free bread that had been fermented for 16 h. On the other hand, Poutanen et al. [86] stated that the fermented food products produced from gluten-free cereals such as rice, maize and sorghum were found in tropical climates. However, the most common LAB species, such as *L. sanfranciscenis*, are found in traditional (wheat, rye) sourdough fermentations as they are not present in fermentations carried out in tropical climates. Instead, more thermophilic species such as *L. fermentum* and *L. reuteri* are commonly found in these fermentations. Nevertheless, it was mentioned in the study of Poutanen et al. [86] that long-time fermentation of dough by selected LAB was shown to be a potential tool to decrease the risk of rye contamination of gluten-free products for coeliac patients.

Dough Structure

Microbial metabolic activity such as synthesis of polysaccharides from sucrose is a simple, economic and a natural way to improve bread quality [29]. Exopolysaccharides, which are bacterial polysaccharides secreted into the environment [13], produced in situ by sourdough lactobacilli affect the rheological properties of dough as well as loaf volume, crumb texture and the handling characteristics of bread [29]. Sourdough LAB affects the microstructure of dough not only by producing exopolysaccharides, but also by producing CO_2 [4] and lowering pH via producing lactic acid [2, 56]. The factors affecting dough structure due to the activity of *L. sanfranciscensis* are classified in Fig. 5.

Exopolysaccharides

Exopolysaccharides (EPS) from LAB may be divided into two classes: (1) homopolysaccharides (HoPS), which are composed of one type of monosaccharide and are synthesized by extracellular glucan- and fructosyltransferases using sucrose as the glycosyl donor; (2) heteropolysaccharides (HePS) having (ir)regular repeating units that are composed of 3–8 carbohydrate moieties are different monosaccharides synthesized intracellulary from sugar nucleotide precursors. HoPS are generally applied to improve the structural characteristics of baked goods and are the exopolysaccharides mostly produced by sourdough lactobacilli [13]. Formation of HePS by cereal-associated lactobacilli has hitherto not been described [101].

It has been reported that one of the key microorganism of sourdough, *L. sanfranciscensis*, produced HoPS such as *fructan* [61, 62, 101]. The fructan produced by *L. sanfranciscensis* LTH2590 was later characterized as *levan* [63, 101]. In addition to EPS, LAB can also produce gluco- or fructooligosaccharides (FOS). *L. sanfranciscensis* has shown the ability to produce the prebiotic FOS 1-kestose [63, 76]. Sucrose is metabolized by *L. sanfranciscensis* by the action of a fructosyltransferase enzyme, and probably a levansucrase, into glucose, kestose and an EPS composed of fructose [63]. Levansucrase, which is responsible for sucrose hydrolysis as well as the formation of kestose and levan as a fructosyltransferase enzyme, is remarkably the only sucrose-hydrolysing enzyme in *L. sanfranciscensis* LTH2590. The polymerization of fructose to a levan chain yields a high-molecular-mass polymer with β- (2 → 6) linkages in the main chain that may be branched with β (2 → 1) linkages [101]. Recent studies demonstrate that the food industry focused on the use of polysaccharides from agricultural plants (see [64] for a review) and the major reasons for the interest in polysaccharides are attributed to their numerous functionalities such as texture modifiers, gelling agents, emulsifiers, thickeners and stabilizers [77]. Hydrocolloids such as pectin, xanthan gum, CMC and β-glucan are widely used in baked goods, especially in gluten-free bakery products, in order to modify rheology and texture [48, 51]. This information led to the possibility to use EPS produced by *L. sanfranciscensis* in bakery products in order to improve their rheological properties. However, there is also growing interest

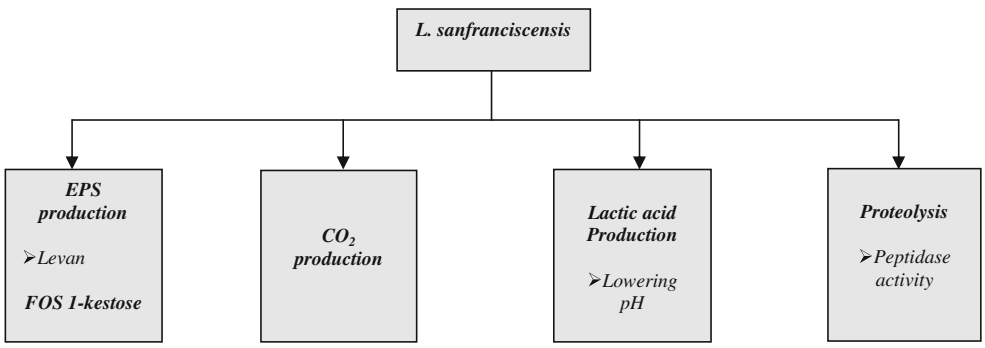


Fig. 5 Effects of *L. sanfranciscensis* on dough microstructure

for polysaccharides with prebiotic attributes, as the food industry realized the developing multifunctional additives that not only provide the desired improvement of the texture but also have additional nutritional properties [63]. Moroni et al. [76] reported that FOS produced by LAB (e.g. 1-kestose produced by *L. sanfranciscensis*), together with the fructan inulin, were well described for their prebiotic effects, whilst improving bread structure.

The fructan from *L. sanfranciscensis* was recently found to positively affect dough rheology and bread texture [2, 61, 100]. Hydrocolloids, such as xanthan or modified cellulose, significantly affect dough rheology and bread texture at levels of 0.1–1 % of the flour base, and levan formed by *L. sanfranciscensis* LTH2590 was shown to affect the rheological properties of wheat doughs at level of 0.1 % of the flour base [100]. Addition of 60 g/kg sucrose to wheat dough resulted in the formation of more than 5 g/kg levan by *L. sanfranciscensis* LTH2590 [101]. Therefore, the amounts of EPS formed during sourdough fermentation by *L. sanfranciscensis* can be assumed to be technologically relevant [100]. Korakli et al. [63] reported that formation in situ of EPS from sucrose resulted in further metabolites such as mannitol, glucose and acetate that may contribute to the improved bread quality. Thus, Tieking and Gänzle [101] stated that polymers produced from lactobacilli 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. EPS-producing LAB is reported by Decock and Cappelle [23] to find industrial application currently in sourdough fermentation to improve dough rheology as well as the textural quality of bread.

CO_2 Production

Increased bread volume has been reported for wheat bread containing up to 20 % sourdough [56]. Formation of CO_2 in sourdough mother sponge as well as the volume and the crust structure of sourdough bread is illustrated in Fig. 6.

Brandt et al. [4] reported that CO_2 formation by *L. sanfranciscensis* in rye bread fermented with *L. sanfranciscensis* and *C. humilis* was highest at the parameter levels favouring its growth. These parameters may be indicated as a high pH (4.5–5.0), a low amount of inoculum (0.1–1 %), a low NaCl concentration (0–1 g/100 g flour) and a temperature of 32 °C. It was also stated that about 50 % of the total CO_2 formed in dough originated from the yeast metabolism, although *C. humilis* contributed to only 3 % of the total cell counts in fermentations under standard conditions. This observation can be attributed to the fact that two moles of CO_2 per hexose are formed by *C. humilis*, compared to one CO_2 per hexose from *L. sanfranciscensis.*

Although most of the obligatory heterofermentative lactobacilli metabolize pentoses, *L. sanfranciscensis* strains do not metabolize. They metabolize hexoses and produce lactate, ethanol and CO_2 as the major products of hexose metabolism, unless the existing co-substrates enable the regeneration of reduced cofactors [40].

Dal Bello et al. [15] investigated the gaseous release and development characteristics of dough samples fermented by *L. plantarum* and *L. sanfranciscensis*. They stated that the addition of sourdough, either fermented by *L. plantarum* FST 1.7 or by *L. sanfranciscensis* LTH 2581 caused a distinct change in the properties of the wheat bread dough. The results of the rheofermentometer tests showed that sourdough improved the gas-holding capacity and the dough development characteristics. It was also stated that the positive effect of an increase in specific loaf volume was observed in sourdough breads when compared with the chemically acidified and non-acidified dough. Gobbetti and Corsetti [45] reported that *L. sanfranciscensis* had a positive influence on yeast leavening and CO_2 production. As it was stated before, an increase in the total CO_2 had been observed when associated with *S. exiguus*M14.

Acidification

The positive effect of sourdough in bread volume has been linked to better gas-holding capacity of gluten in acidic dough containing sourdough. However, increasing acidity may lead to a stepwise degradation of gluten proteins and thus may result in softer, less elastic dough with poorer gas-holding capacities. Accordingly, the acidity level of sourdough must be carefully controlled in order to obtain bread having increased volume [59].

Incorporation of sourdoughs in wheat bread making influences the viscoelastic behaviour of doughs as well as the gluten proteins due to the drop in pH value caused by the organic acids produced. Dough stability has been observed to decrease when the dough was prepared with the addition of sourdough. On the other hand, dough consistency was unchanged when the sourdough was fermented by a heterofermentative culture [56]. The acidification of the sourdough and partial acidification of the bread dough impact on structure-forming components like gluten, starch and arabinoxylans. The swelling of gluten in acid is a well-known effect. Furthermore, acids strongly influence the mixing behaviour of doughs whereby doughs with lower pH values require a slightly shorter mixing time and have less stability than normal doughs [2].

It was mentioned by De Angelis et al. [18] that *L. sanfranciscensis* usually established mutualistic associations with sourdough maltose-negative yeasts and contributed to the bread rheology. Decock and Cappelle [23] stated tha t



Fig. 6 Mother sponge and sourdough breads

small blisters appeared on the surface of the San Francisco bread dough due to the high acidity during fermentation. After the dough is baked using a lot of steam, a very crispy reddish crust is obtained showing the typical fish eyes.

Proteolysis

Arendt et al. [2] stated that the changes in dough rheology and bread texture are the consequences of proteolysis as well as the improvement in flavour. The use of sourdough in bread making requires a limited extent of proteolysis in order to prevent extensive gluten degradation whilst ensuring a sufficient liberation of amino acids for flavour precursors [41]. Pepe et al. [83] also reported that proteolysis can influence rheological parameters, viscosity and gas retention. They have also stated that the degradation of gluten, as a result of the proteolytic activity of the starter culture, affected the viscoelastic properties of the dough with a loss of elasticity. As a consequence, the dough became softer with a reduced capacity to retain CO_2 and an increase was observed in the crumb density of the baked dough. Di Cagno et al. [25] stated that the proteolytic activity in bread making regulates the physical properties of the dough; dough becomes more extensible and develops at a faster rate. They have analysed the textural properties of sourdoughs by using Brabender farinograph and extensograph; and the results have demonstrated that the combination of sourdough and enzymes promoted greater stability and softening during fermentation. De Angelis et al. [18] characterized the peptidase activities of *L. sanfranciscensis* strains. They reported that gluten contains 14.2 % of proline residues and proteolysis during sourdough fermentation depends strictly on the activity of specific peptidases which hydrolyse peptide bonds where

proline is located at the different positions as a potential substrate. On the other hand, Vermeulen et al. [105] carried out a study in order to characterize the proteolytic system of *L. sanfranciscensis* and reported that *L. sanfranciscensis* DSM 20451 did not have proteolytic activity which contributed to the hydrolysis of proteinaceous substrates. *L. sanfranciscensis* needed peptides, which originated from protein hydrolysis by the endogenous flour proteinases, for growth during sourdough fermentation. Metabolism of peptides by *L. sanfranciscensis* may result in the accumulation of smaller peptides, amino acids and amino acid metabolites which contribute directly or indirectly to the characteristics of sourdough bread in case they are not necessary for the growth of the bacteria.

Antimicrobial Properties of Sourdough

The antimicrobial activity of sourdough arises from lactic acid [29], acetic acid [56], CO_2 [29], diacetyl, ethanol, hydrogen peroxide, [13, 73], bacteriocins [11, 45, 75], antifungal compounds such as fatty acids [13] produced by lactic acid bacteria during fermentation. This activity of LAB offers a trend for the baking industry to prevent the undesired microbial activity in bread. During storage of bread, several different physicochemical (e.g. staling, firming) and microbiological changes (e.g. ropiness, mould growth) occur, lowering the quality of bread. The crumb becomes hard, the bread crust changes from crispy to leathery and the characteristics and favourable bread flavour disappears. All these changes are characterized as the *staling* process. Bread firming is the main characteristic of the staling process during storage. It is generally believed that bread firming is mainly caused by recrystallization of the starch fraction, involving amylopectin chains. Starch retrogradation (recrystallization) is considered to be one of the key factors contributing to staling in addition to other factors such as changes in gluten functionality, moisture migration and the glass-rubbery state of bread polymers. In addition to the effects of retrogradation, following the staling process, within a few days the bread might be spoiled due to the contamination and growth of moulds on the surface or development of rope in the bread crumb caused by *Bacillus* ssp [30, 53, 56]. The addition of sourdough retards the staling process of bread [9, 73], prevents the bread against ropiness [73, 103] and prolongs the mould-free period of bread during storage [45, 56].

Contribution of *L. sanfranciscensis* to Shelf-Life of Sourdough Bread

The production of antibacterial substances by *L. sanfranciscensis* may be related to its predominance and may contribute to the stability of sourdough products [45]. Due to these antibacterial substances, sourdough fermentation with *L. sanfranciscensis* plays a role in the prevention of mould contamination and rope spoilage. Additionally, *L. sanfranciscensis* also retards the staling rate of the sourdough bread due to its amylolytic activity.

AntiMould Activity

Microbial spoilage by moulds still remains responsible for huge economic losses in the bakery industries. The most common spoilage fungi from bakery products belong to the genera *Penicillium, Aspergillus, Monilia, Mucor, Endomyces, Cladosporium, Fusarium* and *Rhizopus* [91]. Today, several alternatives are applied in order to prevent or minimize microbial spoilage of bread; for example, addition of propionic acid and its salts [15, 42, 94], ethanol, sorbic and benzoic acids [91], lactate and acetate [113] is used as the chemical preservatives. Corsetti and Settanni [13] stated that *L. sanfranciscensis* had the ability to produce a mixture of acids containing caproic acid, which was the organic acid with the highest antimould activity. Moroni et al. [76] mentioned about caproic acid produced by *L. sanfranciscensis* CB1, together with a mixture of acetic, formic, propionic, butyric and n-valeric acids, played a key role in inhibiting *Fusarium, Penicillium, Aspergillus* and *Monilia* growth in bread. It was mentioned by Gobbetti and Corsetti [45] that a mixed culture pre-ferment of lactic and propionic acid bacteria had been used in breadmaking in order to produce propionic acid for its antimicrobial properties. De Muynck et al. [20] reported that the antifungal activity of *L. plantarum* and *L. sanfranciscensis* was due to the specific organic acids they produced, whereas most of the LAB showed antifungal activity due to the production of antifungal protein or proteinaceous compounds. Dal Bello et al. [15] investigated the antifungal activities of *L. plantarum* and *L. sanfranciscensis* against *Fusaria* and found out that *L. plantarum* FST 1.7 performed inhibitory activity against *Fusaria*, whilst *L. sanfranciscensis* LTH 2581 was not inhibitory against any of the fungi tested, expect *F. graminearum* with the addition of 20 % sourdough. Ryan et al. [94] reported that the inhibitory activity of *L. plantarum* on *P. roqueforti* was reduced when using traditional sourdough fermented by *L. sanfranciscensis* due to the organic acids produced by *L. sanfranciscensis*; whereas the incorporation of 20 % of *L. plantarum* sourdough into wheat bread demonstrated an increase in shelf-life against common bread spoilage organisms. On the other hand, several studies support that *L. sanfranciscensis* had the largest spectrum of antimould activity among obligately heterofermentative LAB [13, 56, 75].

Besides the production of organic acids, another inhibitory activity of LAB on microorganisms causing mould

spoilage in bread is provided by bacteriocins, which are antimicrobial peptides or small proteins that inhibit microorganisms that are usually closely related to the producer strain. Bacteriocins produced by sourdough lactic acid bacteria have been purified and well characterized with regards to their in vitro activity. *L. pentosus* 2MF8 isolated from sourdough [11] was the first example of a sourdough-derived *Lactobacillus* strain that produced an antimicrobial compound with bacteriocin characteristics and reported to be active under sourdough conditions [11, 13]. Corsetti et al. [11] tested the in situ activity of bacteriocin-like inhibitory substances (BLIS) M30, produced by *Lactococcus lactis* subsp. *lactis* M30 and isolated from unmalted barley, in the sourdough ecosystem. They found out that it had been shown to possess a more potent inhibitory spectrum and activity than sourdough LAB, whilst it did not inhibit certain strains of the key sourdough bacterium *L. sanfranciscensis.* A bacteriocin-like inhibitory substance (BLIS C57) was also isolated from *L. sanfranciscensis* C57 [45].

Rope Spoilage Prevention

Ropiness is a spoilage of wheat bread noticed as an unpleasant odour similar to that of overripe melons, followed by the occurence of a discoloured sticky bread crumb and sticky threads that can be pulled from the crumb. This bread spoilage is caused by heat-resistant strains of *Bacillus*, mostly *B. subtilis* and *B. licheniformis* [13, 73, 103] due to their rope formation. Their heat-resistant spores can survive the baking process, sporulate and multiply in the baked bread [56]. However, rope formation occurs principally in wheat breads that have not been acidified, or in breads with high concentrations of sugars, fat or fruits [13]. Martínez-Anaya [71] reported that *L. sanfranciscensis* had some antimicrobial properties, which are considered active towards *B. subtilis* but not against sourdough yeast and moulds. Valerio et al. [103] demonstrated that the sourdough *L. plantarum* and its metabolites such as lactic and acetic acids, which act like calcium propionate (0.3 %), prolonged the *Bacillus* free shelf-life to 7 days at 30 °C. Menteş et al. [73] also reported that the rope spoilage caused by *B. subtilis* might be prevented by the activities of *L. plantarum* LMO25 or *L. alimentarius* LMO7, in case the addition amount of sourdough was 20 %. On the other hand, Zhang et al. [113] indicated that the addition of 20 % of sourdough was not suitable for practical applications as high levels of propionate and acetate in bread dough inhibited the activity of baker's yeast. Hansen [56] stated that the addition of 15 % sourdough was more efficient as the strains of rope-producing *Bacillus* were effectively inhibited by sourdough fermented by *L. sanfranciscensis, L. brevis, L. maltaromicus* or by three different strains of *L. plantarum.* In this study, *B. subtilis* tended to be inhibited if the TTA (total titratable activity) value of sourdough was more than 10 and when the pH of the bread crumb was below 4.8.

Staling Rate of Sourdough Breads

The staling rate was mostly influenced if the starter culture had amylolytic activity (*L. amylovorus* or a genetic modified strain, *L. sanfranciscensis CB1 Amy*) [56]. *Amy* gene was expressed in *L. sanfranciscensis* CB1 in the study Gobbetti et al. [44] carried out. The expression of the α-amylase activity in *L. sanfranciscensis* could potentiate the fermentation ability of this strain, reduce the competition between LAB and yeasts for the soluble carbohydrates of the flour and have a positive role in reducing the staling of baked sourdough products.

Rizzello et al. [91] reported that the addition of freeze-dried sourdough fermented (*L. plantarum* LB1 and *L. rossiae* LB5) wheat germ to bread dough delayed staling of the bread. Furthermore, Hansen [56] stated that the rate of starch retrogradation was not influenced if the acidification was rather low, whereas a standard sourdough fermented by *L. sanfranciscensis* 57, *L. plantarum* 13 and *S. cerevisiae* 141 was able to retard the staling rate. Corsetti et al. [9] stated that sourdoughs with the lowest pH and highest TTA had been proven to be the most favourable for producing a bread with highest volume, good crumb grain and lowest rate of staling during storage. When compared with bread produced by yeast alone, souring delayed both bread firmness and staling. However, LAB strains that did not differ greatly in acidification properties but which demonstrated proteolytic and amylolytic activity such as *L. sanfrancisco* CB1 and *L. plantarum* DC400 had completely different effects on bread shelf-life. The production of organic acids, the bacterial hydrolysis of starch and proteolysis of gluten subunits is the desired properties of LAB in order to retard staling.

Conclusions

Sourdough with *L. sanfranciscensis* is used in the production of many bakery products such as San Francisco bread, rye/wheat breads, *Panettone* cake, brioches, *Pugliese* bread, *Pandoro* cake, *Colomba* cake and many other traditional sourdough breads produced in several countries. It is believed that sourdough fermentation with *L. sanfranciscensis* might lead to the productions of alternative functional bakery products for coeliac patients, for diabetics and for the people having allergy to baker's yeast. As well as leading to the production of functional bakery products, *L. sanfranciscensis* also contributes to the flavour

characteristics, shelf-life and dough rheology/last product texture via its metabolic activities. The contribution of *L. sanfranciscensis* to the characteristics of bakery products during sourdough fermentation due its metabolic activities is demonstrated in Fig. 7. Lactic acid fermentation, and thus acidification and CO_2 production, proteolysis, synthesis of volatile compounds, antimould and antiropiness are the main metabolic activities belonging to *L. sanfranciscensis* during sourdough fermentation. Furthermore, concerning the endogenous factors existing in cereal-based raw materials and the changing process parameters, these metabolic activities significantly determine the characteristic properties of sourdough fermented bakery products. Due to the production of several volatile compounds such as ethyl acetate, alcohols (ethanol, 1-propanol, 2-methyl-1-pentanol, 1-heptanol and 1-octanol), aldehydes (3-methyl-1-bytanal, heptanal, trans-2-heptanal, octanal and nonanal) and acetic acid, *L. sanfranciscensis* contributes to the specific flavour development of bakery products. Its ability to produce EPS, CO_2, lactic acid (pH lowering action) and also its peptidase activity significantly influences the microstructure of dough and the textural properties of the last products. Studies have demonstrated that sourdough fermentation with *L. sanfranciscensis* affects the rheological properties of dough as well as loaf volume, crumb texture and the handling characteristics of bread. On the other hand, it is also believed that EPS produced by *L. sanfranciscensis* may find the opportunity to be used in bakery products (especially in gluten-free products) instead of hydrocolloids in order to improve the dough rheology. Thus, it may be possible to meet the consumers' demand for a reduced use of food additives, whereas the costs of the production of bakery products also decrease. Moreover, sourdough fermentation with *L. sanfranciscensis* improves the shelf-life of bakery products via its antimould and antiropiness activities due to the production of BLIS and specific organic acids and via its amylolytic activities leading to retardation in the staling rate of bakery products.

According to the studies carried out, the addition of 15 % sourdough fermented by *L. sanfranciscensis* into dough formulation effectively inhibited the strains of rope-producing *Bacillus* [56], whilst 5–10 % sourdoughs fermented by *L. sanfranciscensis* is preferred in odour and taste [88]. Despite the numerous advantages, *L. sanfranciscensis* sourdough fermentation provides, sourdough bread production is a challenging process due to the need for long-time fermentation. However, as it has been stated before that a pasteurized San Francisco sourdough in liquid form is currently available on the market, it is possible to produce breads with the specific full flavour and also to shorten the fermentation time via the addition of more yeast using this liquid sourdough. Thus, a San Francisco bread having most of the characteristics of the traditional product may be produced in less than 3 h [23]. The possibility to produce sourdough products fermented by *L. sanfranciscensis* in industrial scale will lead to the alternative functional bakery products having more bioavailability and fewer additives for all consumers, as well

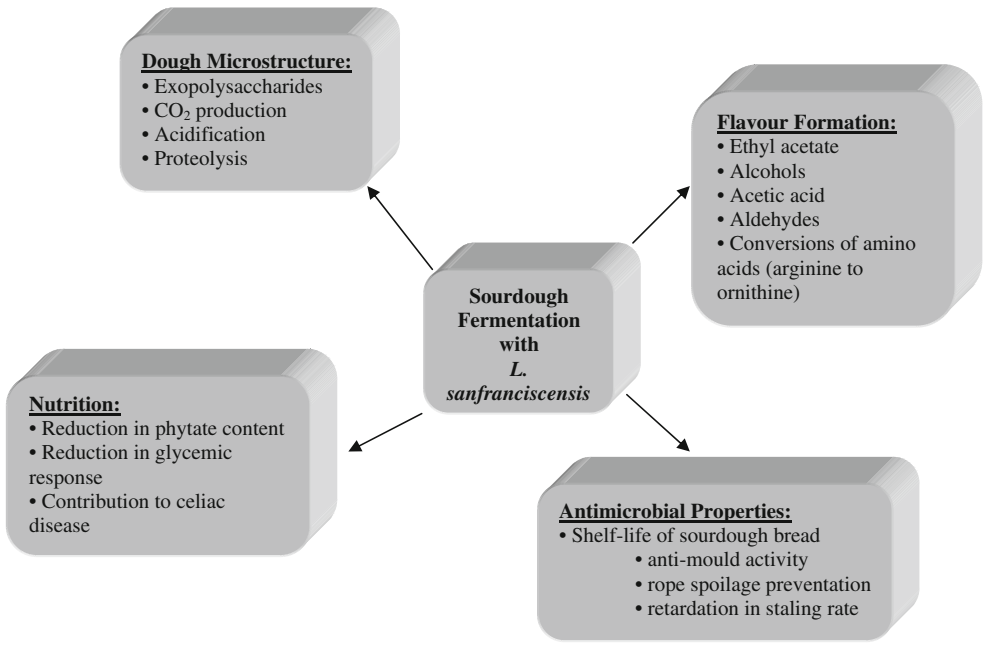


Fig. 7 Contribution of sourdough fermentation with *L. sanfranciscensis* to dough/bread characteristics from several aspects

as the coeliac patients, diabetics and for the anti-*Saccharomyces cerevisiae* antibodies (ASCA).

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