Sourdough and Its Effect on Bread Properties

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Contributor: Ricardo H. Hernández-Figueroa , Emma Mani-López , Enrique Palou , Aurelio López-Malo

Sourdough is a key component in traditional and artisanal bread making. It imparts unique flavors and textures to bread, which are highly sought after by consumers. Accumulated research shows the performance of sourdoughs as an alternative to improve the organoleptic characteristics of bread and its shelf life.

sourdough lactic acid bacteria bread quality antifungals shelf life

1. Introduction

Bread is one of the most consumed fermented foods in the world and is an important part of the food base of many countries ^[1]. In general, bread is made by baking a dough previously fermented with yeast and, in some types of bread, other microorganisms that give important characteristics and quality attributes to this product. However, fresh bread has a short shelf life, governed mainly by physicochemical alterations known as aging and mold growth on the product's surface. To improve the shelf life of bakery products, the baking industry has used the incorporation of synthetic additives and antimicrobial agents, which have been shown to have the ability to reduce both the aging process of bread and the growth of molds on the surface of baking products. However, consumer demand for natural products or products without synthetic additives has generated the search for new "natural" alternatives that help replace these synthetic additives and maintain the shelf life of bread ^{[2][3]}.

One of the oldest ways of bread leavening is sourdough fermentation, converting cereal flour into attractive, tastier, and more digestible products ^{[1][4]}. An alternative approach that has attracted the attention of the scientific community in recent years is the use of sourdough to leaven various bakery products, as well as to incorporate it into the formulation of other products, which creates research opportunities in the use of sourdoughs and their fermentation products with very diverse applications and approaches ^{[1][5]}. An alternative that has attracted attention in recent years is to use sourdough together with baker's yeast to produce bread at an industrial level and have the benefits of its incorporation in the formulation ^[6]. For many years, sourdoughs have been used in the European and Western regions to make bread ^[2] since it has been observed that incorporating them helps maintain the product's organoleptic characteristics and delays the growth of molds on the product's surface. This fermented dough has a complex microbiome that mainly includes lactic acid bacteria and yeasts ^[4]. It has been shown that during the formation of sourdoughs, compounds such as organic acids, peptide compounds, and exopolysaccharides are formed, which help maintain the shelf life of the product, preserving the quality characteristics of the bread and reducing the rate in the growth of molds on bread surface ^{[3][8][9][10][11]}. Likewise, in recent years, the ability of aqueous extracts from fermented sourdoughs to inhibit the growth of the main molds that

spoil bread has been demonstrated, thus generating promising alternatives as natural antimicrobials in bakery products [11][12][13][14][15].

Bread is highly perishable due to its composition, leading to a quality decline from physicochemical and microbiological changes after baking. Post-baking cooling results in moisture loss, affecting crumb and crust texture. Over time, starch retrogradation and moisture loss increase hardness. Mold growth on the bread surface poses a significant economic challenge for the baking industry ^[16].

Molds proliferate easily in bread because it is rich in carbohydrates (70–80 g carbohydrates/100 g bread), with water activity values between 0.94–0.97 and pH values around 6, which are optimal conditions for mold growth. Contamination of bakery products with mold spores, which are naturally found in the bakery environment, has been shown to occur after the baking process, during cooling, slicing, and packaging of the product, these last stages being the determinants to define the microbiological quality of these products ^{[16][17]}.

Penicillium and *Aspergillus* are common molds that cause bread spoilage due to their ability to thrive in diverse conditions, produce numerous spores, and exist in the environment. Notable species include *Penicillium roqueforti, P. paneum, P. corylophilum, P. chrysogenum,* and others, leading to blue-green spots on bread. *Aspergillus* species such as *A. chevalieri* and *A. niger* cause colored spots, ranging from green to black, with some producing yellow pigments. Additional filamentous molds like *Rhizopus, Mucor,* and *Neurospora,* while present in various baking products, are less significant than *Penicillium* and *Aspergillus* ^{[16][17]}.

Bacteria such as *Serratia marcescens* and *Endomyces fibulger* can cause red or white stains on bread. Ropy bread, characterized by yellow color and sticky viscosity, results from specific strains of *Bacillus subtilis*. However, bacterial growth on bread is infrequent compared to mold growth, leading the baking industry to prioritize the elimination of mold contamination ^{[17][18]}.

The baking industry, facing substantial economic losses from mold spoilage of bread, is increasingly developing preservation methods. Propionic, sorbic, and acetic acids, and their salts, are common antifungals for mold prevention ^[14]. In response to consumer demand for "natural" additives, research explores new compounds to enhance the shelf life of baking products. Utilizing sourdoughs or their aqueous extracts in bread formulation is considered an alternative, with numerous studies describing their effectiveness as antifungals.

2. Sourdoughs

Sourdough is one of the oldest examples of natural starters, mostly used for making fermented bread as an alternative to baker's yeast and chemical leavening. The term "sourdough" can vary in different countries and languages. However, the different linguistic expressions capture the idea of a natural leaven or sourdough starter, such as in French: *levain*, Italian: *lievito madre*, German: *Sauerteig*, Spanish: *masa madre* or *levadura madre*, Portuguese: *fermento natural*, Dutch: *zuurdesem*, and many others. Sourdoughs are the result of spontaneous fermentations that are obtained when flours from different grains (mainly wheat or barley) are mixed with an

adequate amount of water and maintained under conditions of temperature and time favorable for the growth of microorganisms inherent to the flour or intentionally inoculated ^[19]. In many countries worldwide, sourdoughs continue to be used as part of their formulation due to their unique texture and flavor characteristics in the final product ^[20]. During sourdough fermentation, metabolite production and chemical reactions occur, giving the product distinctive characteristics and a high sensory quality ^[5]. Likewise, in recent years, the use of sourdough in the baking industry has increased significantly due to the discovery of nutritional improvements, such as the enhancement of the bioavailability of minerals, the production of peptides with antioxidant activity, and the preservative effects on bread, which improve the shelf life ^{[3][21]}.

Microbial analyses and investigations of sourdough microbiota have shown that two types of microorganisms coexist within this ecosystem: lactic acid bacteria (LAB) and yeasts ^[22]. A majority of sourdoughs that are used in bakeries as sole leavening agents include *Fructilactobacillus sanfranciscensis* (formerly *Lactobacillus sanfranciscensis*), which is one of the dominant fermentation microbes ^{[23][24]}. In sourdoughs fermented to achieve high acidity levels, *Limosilactobacillus* and *Lactobacillus* species typically dominate ^[24]. In most analyses, the LAB commonly isolated from sourdoughs are of the *Lactobacillus* genus, whereas *Saccharomyces* spp. and *Candida* spp. have mostly been isolated and identified as yeasts. The biodiversity of both LAB and yeasts in sourdoughs mainly depends on their type and production process ^{[25][26]}. De Vuyst et al. ^[27] provide the most recent comprehensive compilation of lactobacilli in sourdoughs (more than 700) used for bread making.

3. Sourdough and Its Effect on Bread Properties

It has been observed that the fermentation processes of sourdoughs play a significant role in improving the bread's organoleptic attributes. The compounds formed in lactic acid fermentations and their concentration are directly related to improving bread's flavors and textures ^{[3][28][29]}.

3.1. Texture

In general, sourdough fermentation affects dough rheology on two levels: in the sourdough itself and in dough that has had sourdough added to it ^[3]. Various studies have assessed the effect of sourdough on bread formulation and the resulting bread texture. In general, the addition of sourdoughs decreases the resistance to extension, reduces the elasticity of the dough, and increases the degree of softening, obtaining a crumb with greater CO_2 retention capacity (a greater number of alveoli of a larger size), a firmer crust, and better texture. These changes, both in the structure of the crumb and in the dough, have been attributed by several authors to the proteolysis of gluten proteins caused primarily by the increase in acidity due to the production of organic acids during the sourdough fermentation ^{[8][29][30][31][32][33][34][35]}.

Many LAB associated with sourdoughs, especially the *Lactobacillus* genus, produce a variety of exopolysaccharides (EPS) and oligosaccharides during the lactic acid fermentation of sourdoughs. These compounds have been shown to have the ability to function as hydrocolloids and thus improve the texture properties of bread during storage ^[3]. Torrieri et al. ^[34] recorded that adding EPS-producing LAB to sourdough

significantly improved the texture during bread storage, further to gluten proteolysis. Besides, they observed that bread had a higher moisture content and better mechanical properties during storage, which may be an alternative to reducing the aging process of bread ^[34].

3.2. Flavor

Sourdough bread has a complex flavor profile influenced by the compounds generated during microbial fermentation ^{[36][37][38]}. Odors and flavors are generated in the sourdough mainly due to enzymatic and microbial processes during the fermentation of these doughs. These compounds belong to different chemical classes: aldehydes, acids, alcohols, ketones, esters, and pyrazines. The flavors and odors from the raw materials are insignificant compared to those generated during fermentation. However, other aromatic and flavor compounds are produced from lipid oxidation processes and Maillard reactions ^[28].

Two categories of flavor (taste and aroma) compounds are produced during the lactic acid fermentation of sourdoughs. The first of them includes non-volatile compounds, such as organic acids (lactic, acetic, phenyllactic, and phenylacetic acid) that LAB produce and volatile compounds that include alcohols, aldehydes, ketones, esters, and sulfur compounds, which are formed during the fermentation and baking stages of bread [3][28]. The generation of aromatic and flavor compounds is closely related to LAB type, temperature, pH, and moisture content since they directly influence the metabolic activity of the microorganisms ^[3]. Mantzourani et al. ^[38] reported that sourdough (fermented with Lb. paracasei K5) improved bread's sensory properties related to the detected aroma volatiles and consumer preference. Warburton et al. [36] characterized the volatile organic compounds in the crumb of 12 sourdough breads. The authors attributed the observed differences to the culture identified if the fermentation activity was dominated by yeast or the different classes of LAB. The authors associated the profiles in three clusters. In Cluster 1, volatile organic compounds (ethanol, 3-methyl-1-butanol, phenylethanol, 2-methyl-1propanol, acetaldehyde, and 2,3-butanedione) predominated, along with increased production of lactic acid, indicating the activity of yeast and homofermentative or facultative heterofermentative LAB. Cluster 2 was associated with acetic acid, acetate esters, and acidity, revealing that obligate heterofermentative LAB predominates. Fermentation of lipids (production of aldehydes and lactones) related to yeast fermentation activity was classified in Cluster 3.

Siepmann et al. ^[29] observed sourdoughs made with *Lb brevis* and *Lb. plantarum* and with the fermentation temperature changed from 28 to 35 °C. They reported the formation of furfurals, compounds associated with almond aromas in pieces of bread incorporating doughs fermented at 35 °C. On the other hand, pieces of bread made with sourdough fermented at 28 °C did not show the formation of these compounds, demonstrating that the fermentation temperature in the sourdough is important for forming volatile compounds that affect the bread's flavor. Also, they verified that the type of LAB significantly influences the generation of final flavors in the bread. These researchers observed that sourdoughs fermented with *Lb. reuteri*, *Lb. plantarum*, and *Lb. amylovorus* generated a greater diversity of flavor and aroma compounds in the bread than in loaves of bread to which sourdoughs fermented with *Lb. brevis* and *Lb. plantarum* had been added. Xu et al. ^[39] investigated the influence of different starter cultures (*Kazachstania humilis*, *S. cerevisiae*, *Wickerhamomyces anomalus*, *Lb.*

sanfranciscensis DSM20451T and Lactobacillus sakei LS8, in various combinations) on sourdough wheat bread volatiles. Using headspace solid-phase microextraction and gas chromatography/mass spectrometry analysis (SPME-GC/MS) established that sourdough bread fermented with a combination of lactobacilli and yeast had a more complex profile of volatiles (particularly concerning esters). Finally, Siepmann et al. ^[29] determined that both the starter culture and the sourdough fermentation temperature significantly affect bread's final aroma and flavor.

Various investigations have reported different sensory analyses to determine the acceptability of breads formulated with sourdoughs. Sourdough fermentation is known for its ability to produce complex and desirable flavors ^[40]. Understanding sourdough fermentation's microbial dynamics and metabolic pathways ^[41] can lead to new and unique bread flavors and aromatic profiles. Adding 20 to 30% sourdough to the bread formulation has increased the acceptability of both texture and flavor compared with bread made exclusively with yeast. These results demonstrate that the aromatic compounds formed during the fermentation process of sourdoughs help to improve the sensory acceptability of bakery products ^{[8][13][33][38][42]}.

4. Sourdough Effect on the Bread's Shelf Life

It has been shown that LAB produce bioactive compounds with antimicrobial capacity during the lactic acid fermentation process. Substances such as short-chain fatty acids, peptides, diacetyl, hydrogen peroxide, and organic acids have been reported with the potential for inhibiting both pathogenic bacteria and important molds in foods ^{[26][43][44][45][46][47][48][49]}. Some of these compounds have been identified and isolated from sourdoughs, which are also related to the antifungal activity in bread when sourdough is used as an ingredient. In addition, an increase in the bread's shelf life was observed.

Various investigations have been carried out on the ability of sourdough, as an additive, to extend the useful life of bakery products ^{[14][15][26][38][50][51][52]}. **Table 1** presents some examples of these investigations carried out in recent years. According to **Table 1**, adding 20% (*w*/*w*) of sourdough to the bread formulation increases the product's shelf life by an average of six days. Garofalo et al. ^[10] observed that the addition of 30% (*w*/*w*) sourdough in the bread formulation considerably increased the shelf life of the product, achieving improvements of 19 to 21 days (**Table 1**). The type of LAB used for the fermentation of the sourdough affects the effectiveness in increasing the shelf life of the bread. Hernández-Figueroa et al. ^[15] reported that bread incorporating sourdoughs fermented with *Lactobacillus (Lactobacillus acidophilus* NRRL B-4495 or *L. casei* 21/1) had a longer shelf life compared to a traditional one. The addition of a poolish-type sourdough (fermented with *Lactiplantibacillus plantarum* NRRL B-4496) inhibited fungal growth in bread for ten days ^[14]. As can be seen in **Table 1**, the sourdoughs fermented with *Lb. amylovorus, Lb rossiae*, and a mixture of *Lb. rossiae* and *Lb. paralimentarius* presented a significant ability to increase the final product's shelf life.

Table 1. Results of improvement in the shelf life of bread with the addition of sourdoughs fermented by different types of lactic acid bacteria (LAB).

| LAB in Sourdough | % Sourdough Addition (<i>wlw</i>) | Improvement in Shelf Life * | Reference |
|--|--|--------------------------------|------------------|
| Lb. plantarum | 20 | 2 days | [<u>12][53]</u> |
| Lb. sanfrancisencis | 20 | 2 days | |
| Lb. amylovorus | 20 | 9 days | |
| Lb. sakei, Pediococcus acidilactici y Pediococcus pentosaceus | 20 | 6 days | [<u>42</u>] |
| Lb. plantarum | 20 | 1 day | [<u>11</u>] |
| Lb. bulgaricus | 20 | 2 days | |
| Lb. plantarum, L. reuteri and Lb. brevis | 30 | 6 days | [<u>54]</u> |
| Lb. rossiae | 30 | 21 days | [10] |
| Lb. paralimentarius | | 8 days | |
| Lb. rossiae and Lb. paralimentarius | | 19 days | |
| Lactiplantibacillus plantarum NRRL B-4496 | 28 | 9 days | [<u>14</u>] |
| Lb. acidophilus NRRL B-4495 | 38 | 14 days | [<u>15]</u> |
| Lb. casei 21/1 | | | |
| Lb. sanfranciscensis | 30 | >25 days | [<u>51</u>] |

In addition to mold growth, bread aging (retrogradation of starch and loss of moisture) is one of the determining factors for the shelf life of baking products. Torrieri et al. ^[34] observed that adding 30% sourdough fermented with exopolysaccharide-forming LAB to the bread formulation reduced the moisture loss rate and affected starch retrogradation kinetics. The results were attributed mainly to the production of organic acids, bacterial hydrolysis (by LAB) of starch molecules, and proteolysis of gluten subunits. Finally, they concluded that adding sourdoughs to the bread formulation could be an alternative to produce bakery products free of synthetic additives.

Sourdoughs may play a significant role in enhancing bread's quality and shelf life. Decades of research have consistently demonstrated that incorporating sourdough into bread formulations positively influences organoleptic attributes, imparting distinct and desirable flavors and textures. The bioactive compounds generated through sourdough fermentation, especially by lactic acid bacteria, possess antimicrobial properties crucial in prolonging bread shelf life.

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