# **Sourdough Bread Fermentation and Probiotics**

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Sourdough fermentation is an ancient technique to ferment cereal flour that improves bread quality, bringing nutritional and health benefits. The fermented dough has a complex microbiome composed mainly of lactic acid bacteria and yeasts. During fermentation, the production of metabolites and chemical reactions occurs, giving the product unique characteristics and high sensory quality. Mastery of fermentation allows gluten levels to adjust, delay starch digestibility, and increase the bio-accessibility of vitamins and minerals.

Keywords: sourdough bread ; microorganism ; enzymes ; probiotics ; fermentation ; microbiome

# 1. Introduction

Sourdough fermentation is a technique that can use several types of flour, such as wheat, rye, or other cereals, and water. The oldest process of sourdough preparation is spontaneous fermentation and acidification due to the local microbiota in a complex interaction, mainly lactic acid bacteria (LAB) and yeasts <sup>[1]</sup>. It is a traditional fermentation process used for various foods, especially baked goods, improving nutritional and health benefits <sup>[2]</sup>.

Probiotics are safe for human consumption and can produce metabolites that positively influence gastrointestinal <sup>[3]</sup> and bone health diseases <sup>[4]</sup>. Beneficial effects in eczema <sup>[5]</sup>, allergies <sup>[6]</sup>, respiratory tract infection <sup>[7]</sup>, obesity <sup>[8]</sup>, and cognitive and mental health are also described <sup>[9]</sup>. Some LAB and yeasts present in sourdough fermentation are considered and presumed to be probiotics <sup>[10]</sup>.

# 2. Sourdough Fermentation Types: Inoculum and Technology Processes

Sourdough fermentation can be classified according to the inoculum (types II, III, and I) and the technology process (types 0, II, III). According to inoculum, type I uses the microorganisms present in the dough, which results in spontaneous fermentation and utilizes back-slopping to propagate the sourdough microbiota. This process is typical in artisan bakeries and homes. Control parameters include temperature, number, and time of back-slopping in artisan bakeries. Type II inoculums are frequently utilized in industrial processes, where a starter culture is added to sourdough fermentation according to the objectives and results desirable in the final process. Type III is a hybrid and combines type II by using a starter culture with type I for propagating the sourdough with back-slopping. Type III is common in artisan bakeries and industrial ones [11].

According to the production and process, sourdough fermentation can also be classified into four major types. The simple type 0 fermentation starts with a flour–water mixture and is allowed for a limited time. In type 0 fermentation, bioactive molecules and organic acids (lactic and acetic acids) are produced, lowering the pH (pH~4). Still, it is controversial whether this fermentation is a true sourdough. The time limit is insufficient to produce other characteristic products of sourdoughs and is more known as sponge dough <sup>[11]</sup>.

Type I is a traditional method for preparing sourdough and consists of spontaneous flour–water in a flour–water mixture at an ambient temperature of less than 30 °C for 24 h or less and back-slopping frequently. This fermentation increases the quality of the final baked good  $\frac{12}{2}$ .

Type II is when a starter culture initiates the fermentation process and is used to develop desirable characteristics for the baking industry. It is a sourdough in liquid form with controlled parameters <sup>[12]</sup>.

Type III can be dried or lyophilized. It is preferable in an industrial bakery because it has a higher stability quality than fresh sourdough  $\frac{[12]}{2}$ . The fermentation temperature is above 30 °C and a single time between 24–72 h  $\frac{[13]}{2}$ .

The fermentation process will change depending on the added materials, such as the addition of naturally rich products in microorganisms: fruits, yogurt, or another material <sup>[14]</sup>. It is observed that it is challenging to classify sourdough fermentation due to some nuances in the process production <sup>[13]</sup>. **Figure 1** summarizes the major types of production and process and their characteristics.

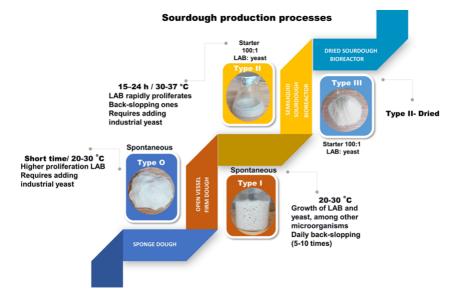


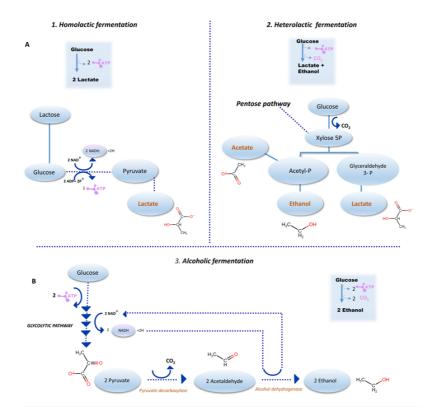
Figure 1. Sourdough production of the four types of processes. Type II and III can be scaled to an industrial level.

### 3. Sourdough Fermentation: Major Pathways

The sourdough fermentation process generates mainly acids, alcohols, aldehydes, esters, and ketones; it is the primary route of volatile organic compounds (VOCs) <sup>[15]</sup>. Sourdough bread has a complex profile, strongly influenced by the compounds generated during fermentation by the diverse array of microorganisms present, mainly yeasts and LAB.

Wheat flour is composed of starch (70–75%), water (14%), proteins (10–12%), non-starch polysaccharides (2–3%), lipids (2%), and soluble carbohydrates such as maltoses, sucrose, and glucose (1.55–1.85%). The starch is degraded into glucose and maltose by flour amylase and glucoamylase by some sourdough LABs. Maltose originates glucose from the enzyme maltose phosphorylase from LAB and alpha-D-glucosidase, a maltase from *Saccharomyces* yeasts. In the dough, the available carbon source is thus maltose, followed by sucrose, glucose, and fructose, with some trisaccharides such as maltotriose and raffinose <sup>[16]</sup>.

The glucose concentration increases during fermentation because other complex carbohydrates are metabolized by LAB and yeasts. Microbial enzymes liberating glucose and galactose after cleavage can ferment the disaccharide lactose. Starting from glucose, homofermentative LAB produces lactic acid through glycolysis, while heterofermentative LAB generates, besides lactic acid,  $CO_2$ , acetic acid, and/or ethanol <sup>[17]</sup>. Two major fermentation routes are found: lactic fermentation (LF) and alcoholic fermentation (AF). In lactic fermentation, the pyruvate molecules formed by glucose oxidation from the Embden–Meyerhof–Parnas or glycolytic pathway are reduced to lactic acid (homolactic fermentation).*Streptococcus, Lactobacillus*, and *Enterococcus* use this route (**Figure 2**A1).



**Figure 2.** Major sourdough fermentation pathways. (A) Lactic fermentation. (B) Alcoholic fermentation. (A1), homolactic fermentation; (A2), heterolactic fermentation. (B3), alcoholic fermentation from yeast. The formation of ethanol and CO<sub>2</sub> from the reduction of pyruvate characterizes alcoholic fermentation.

Another possibility is the pyruvate originating from a mixture of lactate, ethanol, and/or acetic acid through the oxidation of the coenzymes NADH + and H+ by the lactate dehydrogenase and CO<sub>2</sub> from a glucose molecule until ribulose 5 phosphate (heterolactic fermentation). This fermentation is used by genera such as Leuconostoc, Bifidobacterium, Weissella, and some Lactobacillus. Some glycolytic enzymes are missing in these bacteria, and they use the pentose-P pathway to degrade the glucose (Figure 2A2). Classification according to the type of lactic fermentation defines three major Lactobacillus groups: group I: obligate homofermentative lactobacilli. Hexoses are exclusively fermented to lactic acid via the Embden-Meyerhof-Parnas pathway. The bacteria have the enzyme fructose-1,6-bisphosphate aldolase, but phosphoketolase is absent. Due to this, pentoses and gluconate are not fermented. This group includes the species L. acidophilus, L. delbrueckii, and L. salivarius; group II: facultative heterofermentative lactobacilli. Hexoses are fermented to lactic acid almost exclusively via the Embden-Meyerhof-Parnas pathway. The bacteria possess both aldolase and phosphoketolase enzymes and ferment not only hexoses but also pentoses. In the presence of glucose, the enzymes of the phosphogluconate pathway are inhibited. This group includes L. casei, L. paracasei, L. plantarum, and L. curvatus. Organic acids, CO<sub>2</sub>, alcohol, and H<sub>2</sub>O<sub>2</sub> could be produced; group III: is composed of obligate heterofermentative Lactobacilli. They have the enzyme phosphoketolase but not aldolase and ferment sugars in a heterofermentative mode. Hexoses are fermented via the phosphogluconate pathway, producing lactate, ethanol (acetic acid), and CO<sub>2</sub> in equimolar amounts. Pentoses enter this pathway and can also be fermented [18].

After the enzymatic hydrolysis of pentosans and other complex carbohydrates, pentoses such as d-xylose, ribose, and larabinose are liberated in rye and wheat flour. Heterofermentative strains can ferment pentoses through part of the 6phosphogluconate pathway <sup>[19]</sup>. Pentoses and hexoses are simultaneously rather than successively fermented by sourdough Lactobacilli. Lactobacilli are essential during the fermentation of sourdoughs, and they can ferment pentose carbohydrates without producing  $CO_2$  because they have a constitutive phosphoketolase. Facultatively heterofermentative lactic acid bacteria produce the phosphoketolase enzyme in response to the presence of pentoses <sup>[20]</sup>. The contribution of LAB to the flavor of sourdough bread is associated with the production of lactic acid (fresh acidity) and acetic acid (sharp acidity). The conversion of amino acids such as phenylalanine (sweet), isoleucine (acidic), glycine, serine, and alanine (vinegar/sour) to aldehydes and ketones can form additional flavor compounds <sup>[15]</sup>.

Alcoholic fermentation (AF) is characterized by the formation of ethanol and  $CO_2$  from pyruvate reduction (**Figure 2B**). AF is a metabolic pathway of yeasts. In this fermentation, yeast produces gas that promotes dough conditioning and increased volume and imparts desirable aromas and flavors in baked goods. A synergy occurs between the dough and the yeast, and in this research, the rate of  $CO_2$  production is determined by the activities of the glycolytic yeast enzymes. The retention of  $CO_2$  produced by yeast is a function of wheat or cereal flour. It is essential to point out that glucose formed by LAB amylases from amide is a significant source of this substrate for yeasts. Other compounds are volatile organic compounds (VOCs), which arise from this interaction between yeasts and LABs. Diverse aldehydes, alcohols, esters, ketones, lactones, sulfur compounds, furan derivatives, and hydrocarbons are found, conferring flavor and other characteristics to sourdough bread <sup>[21]</sup>. Some VOCs are due to yeast fermentation, particularly of *S. cerevisiae* in mixed sourdough starters, including ethanol, 2,3-butanedione, 2-methyl-1-propanol, 3-methyl-1-butanol, and phenyl ethyl alcohol <sup>[22]</sup>. Other negatively correlated compounds have been associated with the fermentation of homofermentative and facultative heterofermentative LAB, including 2,3-butanedione and acetaldehyde <sup>[23]</sup>.

# 4. Probiotics and Postbiotics in Sourdough and the Impact on Human Health

Probiotics are live microorganisms that confer a health benefit when administered in adequate amounts. In addition, new terms have been used to name microbial metabolites from non-viable cells, including paraprobiotics, parapsychobiotics, ghost probiotics, matabiotics, tyndallized probiotics, bacterial lysates, and postbiotics. However, the last term is being mightily used for the vital concept of promoting health. Postbiotics are non-living microorganisms and is a preparation with inanimate microbial cells or their components that promote host health. Metabolites and cellular structures from microorganisms from sourdough are potential postbiotics [24].

The sourdough microbiota changes with decreasing pH, whereas at the beginning, there is abundant Proteobacteria phylum sourdoughs, which at the end is replaced by Firmicutes  $\frac{[25]}{2}$ , and among yeasts *Saccharomyces cerevisiae* is the most common <sup>[1]</sup>.

A sourdough starter is in essence, a culture of probiotic yeast and lactic bacteria to be added to the fermentation process, besides the native microbiome of the cereals and external contaminations. Starters also can be used to propagate the sourdough as a live fermentation. Here, it is interesting to explore the technological and biological properties of the selected starter associations of bacteria/yeast to improve the quality of fermentation and bread properties.

The microbial production of bioactive peptides, organic acids, exopolysaccharides (EPS), and vitamins are considered the primary metabolites responsible for antioxidant, antimicrobial, and probiotic activities <sup>[26]</sup>. Relative to fermentative processes, many bioproducts are present in sourdough fermentation. Koistinen et al. <sup>[27]</sup> demonstrated 118 bioactive compounds in sourdough fermentation.

One important point is that most probiotics die when the bread is baked at high temperatures. However, most health benefits continue, mostly, not with probiotics at intestinal epithelial cell colonization but in sourdough dough. It is essential to note that active biomolecules could be present in the baked bread. Different conditions, varying temperatures, baking time, and fermentation parameters could influence the heat resistance of some bioproducts, and they could be present as nutrients and fibers such as  $\beta$ -glucan and resistant starch <sup>[28]</sup>.

During the baking process, cell lysis of microorganisms delivers cellular debris that may also have beneficial properties functioning as postbiotics. Remains of microbial cell structures such as peptidoglycan from bacterial cell walls, pili, fimbriae, flagella, cell-surface-associated proteins, cell-wall-bound biosurfactants, and cell supernatants are also postbiotic components that have potential health benefits in the host <sup>[29]</sup>.

Among lactic acid bacteria, one of the most versatile and adaptable is *Lactiplantibacillus plantarum*, with a long history of use in foods and claims of benefits as a probiotic. *Lactiplantibacillus plantarum* strain ITM21B has been used to prolong bread shelf-life due to the production of antimicrobial substances, such as lactic, acetic, phenyl lactic (PLA), and hydroxy-phenyl lactic (OH-PLA) acids <sup>[30]</sup>.

Dietary sourdough bread increases y-aminobutyric acid (GABA) content <sup>[31]</sup>. The y-aminobutyric acid GABA is a nonprotein amino acid synthesized from L-glutamate by the glutamate decarboxylase <sup>[32]</sup>. It is a mammalian neurotransmitter involved in critical regulatory functions, hypotensive properties, anti-depressive effects, diuretic, and antioxidant effects <sup>[33]</sup>. It is present in many medicines and supplements <sup>[34]</sup>. GABA has several physiological functions, include relaxation, sleeplessness, enhanced immunity under stress, increasing the concentration of growth hormone in plasma, preventing diabetes, inhibiting the invasion and metastasis of various types of cancer, anti-inflammatory effects, blood pressure regulation, and antioxidant effects. It is also a hormonal regulator (for review, see Diana et al., 2014 <sup>[35]</sup> and Polak et al. 2021 <sup>[36]</sup>.

A study by Boakye et al. 2022 <sup>[37]</sup>, described that the longer sourdough fermentation time (12 h) caused up to 69%, 69%, and 41% reductions in fructans, raffinose, and ATIs, respectively. Low FODMAPs may be valuable for people with gastrointestinal disorders.

Antioxidant and anti-inflammatory activities of peptides from cooked sourdough breads were described <sup>[38]</sup>, where longer fermentation (72–96 h) increases the production of aromatic compounds with antioxidant activity <sup>[39]</sup>.

LAB and yeast strains were isolated and molecularly identified from traditional Iranian sourdough. Based on total phenol production and antioxidant capacity assessments, all the identified traditional sourdough microorganisms significantly produced phenolic compounds. They showed significant antioxidant capacity improving bread's health benefits and quality [40].

Exopolysaccharides (EPS), such as  $\beta$ -glucan, dextran, and inulin, are metabolites from LAB identified in sourdough fermentation. The  $\beta$ -glucan, for instance, is a prebiotic homopolysaccharide formed by glucose, presenting substantial health benefits, such as stabilized cholesterol levels, anti-inflammatory effects, and benefits for probiotic microorganisms <sup>[41]</sup>. The conclusions reached by Schlörmann et al. <sup>[42]</sup> provide significant insights into the general chemopreventive and prebiotic effects of LAB-generated sourdoughs and bread. In addition, exopolysaccharides are produced by LAB in sourdough, favoring great water retention.  $\beta$ -glucans also contribute to the viscoelastic properties of dough <sup>[41]</sup>. Some of these health benefits and others are summarized in **Table 1**.

Microorganisms	Compound	Benefit	Reference
Probiotics Streptococcus thermophilus, Lactobacillus plantarum, L. acidophilus, L. casei, L. delbrueckii spp. bulgaricus, Bifidobacterium breve, B. longum, and B. infantis	Peptidase	Alfa-gliadin degradation, reduced wheat allergenic	[43]
LAB from sourdough	Gamma-aminobutyric acid (GABA)	ACE-inhibitory activity	[44]
LAB from sourdough	Multifactors	Low-glycemic index in the white wheat bread	[ <u>45]</u>
Lactobacillus reuteri	Exopolysaccharide	Antiadhesive properties, inhibition enterotoxigenic <i>Escherichia coli</i>	[ <u>46]</u>
Lactobacillus brevis with S. cerevisiae var. Chevalieri; L. Fermentum; L. Fermentum with phytase	Higher total phenolic and a lower molar ratio of lactic to acetic acid	Reduce glycemic index	[ <u>47]</u>
L. curvatus SAL33 and L. Brevis AM7	Peptide lunasin	Cancer preventive	[ <u>48]</u>
Bifidobacterium strains	Phytase	Increase iron bioavailability in bread	[ <u>49]</u>
Weissella ciabaria MG1; L. Reuteri VIP, L. Reuteri Y2	Oligosaccharides	Improved nutritional quality of sorghum bread	[ <u>50]</u>
L. brevis	Phytase	Decrease phytate levels, improve mineral bio- accessibility	[ <u>51]</u>
L. Sakei KTU05-6	Organic acids, bacteriocins	Bio-preservative	[ <u>52]</u>

 Table 1. Microorganisms and the compounds they produce, and the health benefits.

Microorganisms	Compound	Benefit	Reference
Weissella confusa LBAE C39-2	Dextransucrase (glycoside hydrolase)	Alfa-glucans/ oligosaccharides or glycoconjugates	[53]
<i>L. rossiae</i> DSM 15814 from sourdough	Vitamin B12, folate, and riboflavin	Nutritional improvement	[ <u>54]</u>
Lactobacillus amylovorus DSM 19280 and Weisella cibari MG1	Glutamate accumulation	NaCl reduction in bread	[55]
Traditional sourdough LAB starter culture	Essential and non- essential amino acids, flavonoids, antioxidant peptides	Nutritional improvement protects against oxidative stress and degenerative disease through phenolic compounds	[ <u>56]</u>
LAB from traditional Austrian sourdoughs	Fructose metabolized/antifungal and anti-bacillus properties	Decrease FODMAPS/ control molds	[57]
<i>Lactobacillus plantarum</i> ZJUFT17 from Chinese sourdough	In mice, decreased: the profile, insulin resistance, lipopolysaccharide, cytokines interleukin (IL)-1β, tumor necrosis factor (TNF)- α	Managing gut microbiota, decreasing pathogenic and pro-inflammatory microbes, and stimulating anti-obesity ones	[ <u>58]</u>
<i>Levilactobacillus brevis</i> TMW 1.211, <i>Pediococcus</i> <i>claussenii</i> TMW 2.340 from breweries	O2-substituted (1,3)- β-D-glucan	Prebiotic effect in bread, improving water binding capacity	[59]
<i>Lactobacillus plantarum</i> ZJUFB2 from Chinese sourdough	Probiotic effect on gut microbiota	Prevent insulin resistance and modulate gut microbiota.	[ <u>60]</u>
Levilactobacillus brevis TMW 1.2112, Pediococcus claussenii TMW 2.340	Dietary fiber, short acid fat chain SCFA, butyrate, propionate,β-glucan	Healthy environment in the colon, chemopreventive	[ <u>61]</u>
Pediococcus pentosaceus F01, Levilactobacillus brevis MRS4, Lactiplantibacillus plantarum H64, and C48	Г-aminobutyric acid (GABA)	Bread from surplus bread with high nutritional value	[62]
Weissella cibaria PDER21	α-D-glucan	Antioxidant properties	[ <u>63]</u>

In addition to the health effects, sourdough brings flavor, aroma, and better texture of bread due to LAB enzymatic processes.

# 5. Enzymes in Sourdough

Enzymes are responsible for several biochemical events in sourdough fermentation. Although some enzymes are present in the cereal, most of them are produced by microorganisms. **Figure 3** shows the sourdough fermentation process and the major enzymes involved.

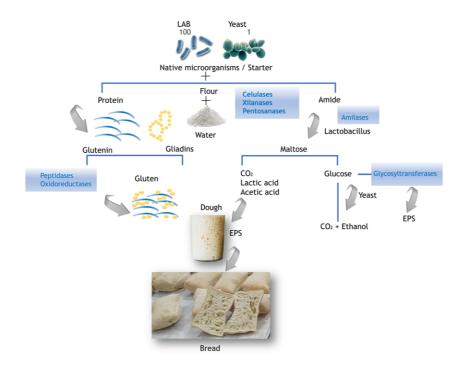


Figure 3. General scheme of bread sourdough fermentation and the major enzymes involved. EPS: Exopolysaccharides.

#### 5.1. Transferases

Glycosyltransferases (EC 2.4) are involved in the biosynthesis of exopolysaccharides in sourdough. It is an enzymatic group produced by the LAB from sourdough microbiota, such as *Lactobacillus*, *Leuconostoc* spp., *Leuconostoc citreum*, and *Weissella* species. Glucansucrases are glycosyltransferases from LAB that split sucrose. The resulting glucose builds EPS-type  $\alpha$ -glucan polymers such as dextran, mutan, alternan, and reuteran. Fructansucrase, found in Gram-negative and Gram-positive bacteria, is another glycosyltransferase that synthesizes levan (levansucrase) or inulin (inulosucrase). The inulosucrase is observed only in LAB <sup>[64]</sup>.

EPS formed during sourdough fermentation influences the dough's viscoelastic properties and improves the texture and shelf-life. Here, EPS can replace hydrocolloids used as bread improvers and meet consumer demands for reduced use of food additives [65].

#### 5.2. Oxidoreductases

Glutathione reductase (EC 1.8.1.7) is the most known enzyme in this group related to sourdough bread properties. It acts on a sulfur group of donors, with NAD+ or NADP+ as the acceptor <sup>[66]</sup>.

Gluten protein presents intra- and intermolecular chains of disulfide bonds between amino acids <sup>[62]</sup>. The glutathione reductase activity, identified in the microbiota of natural fermentation, interferes with these thiol bonds <sup>[68]</sup>. The glutathione reductase deficient mutant strain (*Lactobacillus sanfranciscensis*) produced bread with a softer texture and higher specific volume than bread made with traditional biological yeast and non-mutant *Lactobacillus sanfranciscensis* activity <sup>[69]</sup>.

#### 5.3. Lyases

The glutamate decarboxylase (EC 4.1.1.15) is commonly found in the sourdough environment and acts in L-glutamate's decarboxylation to form  $\gamma$ -aminobutyric acid (GABA) <sup>[70]</sup>.

#### 5.4. Hydrolases

The amylases produced by LAB strains reduce the aging process during bread storage <sup>[71]</sup>. Amylase-producing microorganisms in the sourdough environment are essential in converting starch into fermentable carbohydrates such as maltodextrins, maltose, sucrose, and glucose. LAB are sources of maltose phosphorylase, which generate D-glucose,  $\beta$ -D-glucose, 1-phosphate, and 1,6- $\alpha$ -glucosidase, which hydrolyzes  $\alpha$ -(1–6)-glucooligosaccharides <sup>[72]</sup>. In addition, wheat has  $\alpha$ -amylase,  $\beta$ -amylase, and glucoamylase activity, but at pH < 4.5, only glucoamylase maintains its activity. Microorganisms in the sourdough microbiota produce inulinase ( $\beta$ -2, 1-D-fructan-fructan-hydrolase—EC 3.2.1.7)—a glycosylase that hydrolyzes the  $\beta$ -2.1 bonds of fructose of the fructose polymer, inulin. Inulinase can reduce the number of oligosaccharides, disaccharides, monosaccharides, and fermentable polyols (FODMAPs) in bread <sup>[73]</sup>.

#### 5.4.2. Cellulase, Phytase, and Xylanase for Mineral Bio-Accessibility Improvement in Bread

Endocellulase acts on cellulose and  $\beta$ -glucan substrates, removing insoluble arabinoxylans, contributing to gluten network formation <sup>[74]</sup>. Subsequently, there is a decrease in the hardness of the bread and, consequently, an improvement in the sensory evaluations. Cellulase action may also provide an anti-staling effect, which may be related to alterations in water distribution between the starch–protein matrix <sup>[75]</sup>. The enzyme phytase or myoinositol-hexaphosphate phosphohydrolase is present in plants, bacteria, yeasts, and filamentous fungi. Xylan is the main hemicellulosic component of hardwoods and accounts for approximately 30% of the woody cell wall. Xylanases (EC 3.2.1.8) are enzymes that hydrolyze this polysaccharide. Two mechanisms can summarize the improvement in bread quality due to the addition of xylanase: (i) the removal of arabinoxylans from gluten alters the distribution of water between gluten proteins and arabinoxylans; (ii) pentosan destruction and viscosity reduction effect <sup>[75]</sup>. Xylanase and cellulase showed satisfactory results to facilitate access into the aleurone tissue for the action of phytase, improving iron bio-accessibility <sup>[76]</sup>.

#### 5.4.3. Lipase and the Baking Technology

In baking, using lipase to replace emulsifiers, such as monoglycerides, proved to be an efficient alternative to replace chemical additives  $^{[ZZ]}$ . Sourdough fermentation inhibits the lipase activity due to decreased pH in the system, which is an efficient alternative for neutralizing the lipase activity in wheat germ  $^{[78]}$ .

#### 5.4.4. Peptidase and Implications for the Gluten Network

Gluten structure varies with the cultivar and cultivation conditions <sup>[79]</sup>. It is composed of gliadin ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\omega$ ), glutenin, and polypeptide fractions that combine with gliadin or glutenin <sup>[80]</sup>.

During sourdough fermentation, the partial hydrolysis of gliadin and glutenin proteins occurs because of the acidification and activation of cereal peptidases. In addition, endogenous flour peptidases became activated at a low pH reducing the gluten disulfide bonds <sup>[17]</sup>.

Several studies have focused on LAB peptidases and demonstrated that in different ways, that LAB in sourdough fermentation has a proteolytic activity for gluten hydrolysis <sup>[81]</sup>. *L. rhamnosus, Pediococcus pentosaceus*, and *Lactobacillus curvatus* used gluten as the only source of nitrogen, significantly reducing the allergenic composition of wheat and improving digestibility <sup>[82]</sup>.

# 6. General Regulation for Microbes Used in Sourdough Bread

Searching for rules that maintain food safety is a critical concern due to the growing use of microorganisms as foods or probiotics. At this moment, there is an effort at Codex Alimentarius to harmonize rules for probiotics and the use of microorganisms in food <sup>[83]</sup>.

In the United States, there are rules defined by the Food and Drug Administration (FDA) for the use of microorganisms in food production. It is necessary to qualify as a Generally Recognized as Safe for Use (GRAS) microorganism <sup>[84]</sup>. In Brazil, the biological fermenting mixtures and their microbial content are classified as a supporting and processing agent and are exempted from registration by ANVISA <sup>[85]</sup>. In Europe, since 1997, with the novel food regulation, all food that started to be produced after 15 May 1997 is considered a novel food with specific rules to follow <sup>[86]</sup>. For microorganisms usually found in sourdough and used as a starter, it is challenging to define it as a novel food because sourdough predates 1997 <sup>[87]</sup>.

# 7. Conclusions

The role of microorganisms in sourdough bread has been extensively demonstrated in the literature. Increasing information is arising about the fermentation process and other metabolic routes promoting improvements in quality and adding interesting nutritional, health, and sensory characteristics. Currently, several benefits of probiotic and postbiotics

elements are well established in terms of the bread's properties, such as rheological and organoleptic properties, as well as dough quality and human health.

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