

Review

Sourdough Technology as a Tool for the Development of Healthier Grain-Based Products: An Update

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Abstract: There has been growing demand by consumers for grain-based products with well-balanced nutritional profiles and health-promoting properties. The components of the flours obtained from different grains can be modified or improved at a nutritional level by using sourdough technology, which has gained increasing interest in recent years. Sourdough hydrolyse dietary fibre, reduces fat rancidity, and enables an increase in starch and protein digestibility, as well as vitamin levels and mineral bioavailability. In addition, bioactive compounds are synthesized during fermentation, while components that interfere with the digestion of grain-based products or digestion-linked pathologies, such as gluten sensitivity or gastrointestinal syndromes, are reduced. Finally, it has been observed that sourdough fermented products can play a role in gut microbiota regulation. Thanks to this health-promoting potential, sourdough can stand out among other fermentation processes and opens up a new range of healthier commercial products to be developed. The current review discusses the extensive research carried out in the last 15 years and aims at updating and deepening understanding on how sourdough can enhance the nutritional and health-related characteristics of the different components present in the grains.

Keywords: sourdough; fermentation; nutrition; bread making; microbiota

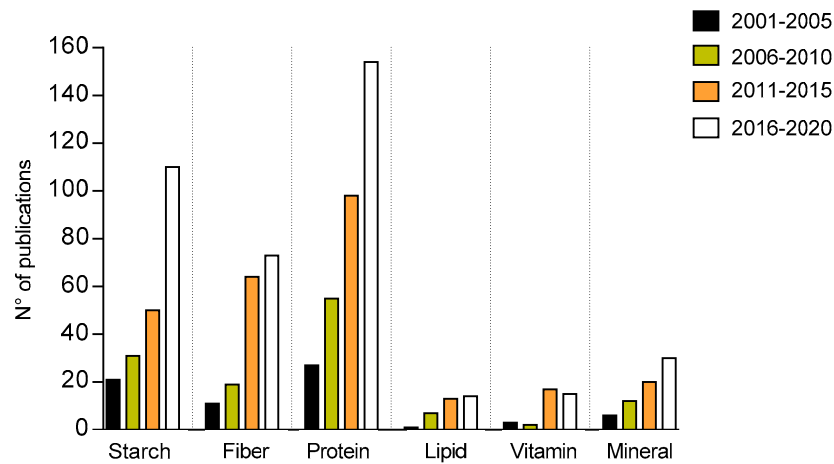
1. Introduction

Sourdough is the result of the fermentation of flour from cereals and pseudocereals or legumes, among others, by the action of the microorganisms present in the preparation [1]. Some sourdoughs can also incorporate added microorganisms. Therefore, sourdoughs can be defined as stable ecosystems composed of the lactic acid bacteria (LAB) and yeasts used in the production of bakery products [2]. Traditionally, sourdough was used as a leavening agent, but today, it is increasingly being used to improve organoleptic characteristics and to reduce the need for additives [3].

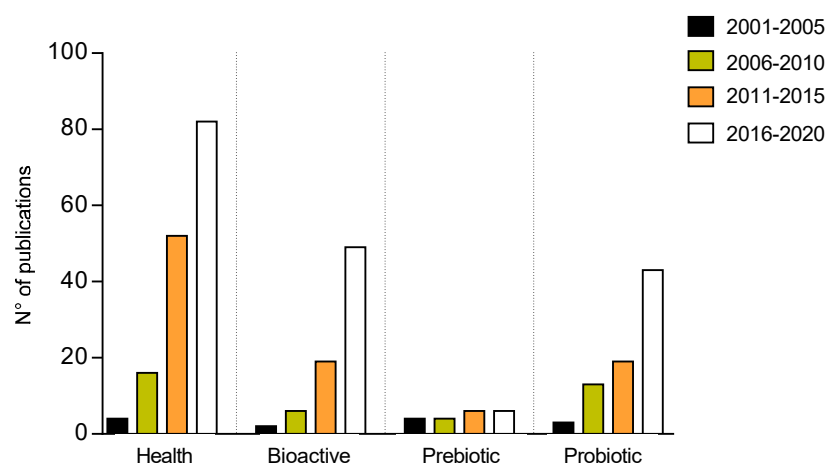
Sourdough provides multiple benefits to the quality of the products obtained and it allows extending of the life of the bakery products [4]. This increased shelf life is mainly due to the lowering of pH, which induces the inhibition of microbial development, and to the decomposition of starch during lactic acid fermentation, resulting in staling delay. The use of sourdough also makes it possible to obtain products with greater aroma and sweetness, due to the hydrolysis processes and the compounds generated in the Maillard reaction during the baking process [5]. However, the application of sourdough can also lead to complications, such as the increased workload required or the difficulty in achieving homogeneous productions [6].

The modification of cereal and grain nutrients and flours by the microorganisms contained in the sourdough to improve bakery products has gained great interest from researchers in recent years.

Consequently, the number of publications addressing the interrelationship between sourdough and the different components of bread has increased significantly in recent years (Figure 1a). Similarly, recently published reviews, such as Sakandar et al. (2019) [7], highlight the processing benefits provided by this technology, focusing on bread making.



(a)



(b)

Figure 1. Number of publications about sourdough over the period 2001–2020, separated in 5-year periods, according to the Scopus database (last accessed 30 June 2020). (a) Publications on sourdough according to the relation with sourdough components. Search: “sourdough AND starch/fibre/protein/lipid/vitamin/mineral”. (b) Publications on the sourdough-health relationship, and functioning components. Search: “sourdough AND health/bioactive/prebiotic/probiotic”.

It has been observed that sourdoughs allow a reduction in gluten content, which may be particularly interesting for lowering the risks of gluten contamination in the case of gluten-free products [8,9]. Even if this does not involve a nutritional improvement, sourdough can contribute as well to improve the organoleptic quality of the gluten-free products, expanding the offer of products suitable for celiac patients or those allergic to wheat [10–12]. However, it is necessary to be cautious of this possibility, since the complete elimination of toxicity for people with celiac disease implies important modifications in the gluten network, affecting gluten functionality and bread quality.

Some studies show that the use of sourdough enables an increase in the sensation of satiety that the breads generate when they are consumed as well as in the postprandial insulinemic response [13]. In addition, other research studies point to a greater digestibility of sourdough-fermented cereal products, compared to yeast-fermented ones [14,15].

Although most sourdoughs are made from wheat flour, for doughs based on legume or pseudocereal flours, it has been shown that this technique reduces the antinutrient content and increases the acceptability of the products. This has also been observed with the incorporation of bran or other by-products [16,17]. Compounds such as fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP), which lie behind disorders such as irritable bowel syndrome (IBS), can be diminished by the action of the fermentation process of sourdough [18,19]. Other innovative approaches include the use of sourdough to reduce the amount of sugar added to bakery products [20] and the ability of sourdough-fermented products to regulate the gut microbiome [21].

Despite reviews concerning the effect of sourdoughs on the various components of flour [22,23], there is still a great potential for improving the health properties of products made from sourdough. This is evidenced by the high number of research articles published in the last 15 years on the topic (Figure 1b). Many of them are based on this greater accrued understanding of microbial ecology, which has enabled a better selection of starter cultures with the use of innovative techniques [1,24,25]. Other articles focus on the nutritional modifications and health-promoting potential of sourdough fermentation, as will be discussed hereafter.

The aim of this review is to update the current understanding of the relationship between fermentation with sourdough, changes in the chemical composition of the products obtained, and the nutritional improvement of cereal and grain products. In addition, we will address the health benefits that the consumption of products with this type of fermentation can entail.

2. Sourdough and Carbohydrates

2.1. Influence of Sourdough Fermentation on Starch Digestibility

Starch digestibility after sourdough fermentation has been the subject of significant research, probably because starch is the main fraction in grains and cereals [22]. This research covers aspects such as glycaemic response or its effect on chronic metabolic diseases, such as type 2 diabetes, obesity, or cardiovascular disease, which have been linked to the high consumption of rapidly digestible starch [26].

Sourdough fermentation reduces the insulinemic response of breads. Nordlund et al. (2016) [27] explain this in terms of the reduced disintegration of breads fermented by sourdough. Furthermore, different studies have shown that the addition of sourdoughs to the baking process helps diminish the glycaemic index (GI) of wheat bread, whether it is made with white, wholemeal, or fibre-enriched flour [28–34]. This is related to the lower pH generated by fermentation before gelatinisation of the starch, as will be explained later. While the consumption of wholemeal bread has been associated with a decrease in glycaemic index as compared with white flour breads, this index is reduced even further when sourdough is used [35,36]. The glycaemic response to bread, however, is specific to each person [37]. The reduced digestibility of starch has been observed not only in bread [38], but also in products such as pasta made from sourdough, due to the higher level of retrograded starch that is inaccessible to enzymatic degradation [39,40]. However, in general, the beneficial effect of sourdough on the GI is attributed to the production of organic acids, especially lactic and acetic acid. These acids represent major fermentation end-products during sourdough fermentation [41]. Lactic acid production by LAB in sourdough-fermented bread reduces postprandial glycaemic and insulinemic responses in healthy adults [14,33,42,43]. The addition of lactic acid is effective to lower the postprandial glucose and insulin responses in humans, when done before heat application, and therefore, before the gelatinisation of the starch, which reduces its bioavailability [44]. In turn, acetic acid produces a delayed gastric emptying rate, but is not required to be present before the gelatinisation of the starch [45].

This suggests that the action of the sourdoughs may depend on the lactic/acetic ratio generated by them. De Angelis et al. (2007) [28] explain that lactic acid is more effective than acetic acid in reducing starch hydrolysis.

In this regard, to reduce starch digestibility, acidification techniques are currently being applied to some products, which promote the interaction between starch and proteins, gluten in particular, and reduce the bioavailability [46]. In the literature, it is stated that the reduction of compounds such as amylase and trypsin inhibitors also facilitates the digestion of products made from wheat flour fermented with sourdough [47]. Another aspect to consider is the increased formation of resistant starch, and the consequent lower digestibility due to the increment of organic acid in the fermentation of the dough [48]. It has been shown that digestibility also decreases if sourdough fermentation is combined with subsequent application of freezing, with reductions in the GI of the products by over 40% [49].

Finally, considering the effect of sourdough over the GI of breads, the type of flour has to be taken into account. Despite GI reduction in wheat breads when sourdough is used, sourdough fermentation showed an increase in the estimated GI of gluten-free breads made with buckwheat flour, quinoa, and teff [34,50]. According to Giuberti and Gallo (2018) [51], this could be because the use of pseudocereal flours, with or without sourdough, does not always guarantee an increase in slowly digestible starch. Moreover, Wolter et al. (2013) [52] suggest that it can be explained in terms of the higher GI of the flours with smaller starch granule size. Nevertheless, studies on gluten-free breads are still limited and should be interpreted considering the processing parameters.

2.2. Interaction between Sourdough Fermentation and Dietary Fibre

Currently, there is a tendency to look for new sources of dietary fibre (DF) as a functional component for the food industry, because of its potential to promote numerous beneficial physiological effects. Dietary fibre contributes to lower blood cholesterol and glucose levels, plays an important role in the health-promoting effect, and there is evidence of a strong association between chronic disease and obesity and a poor dietary fibre intake [53]. However, these effects depend on the type of fibre. The whole grain dietary fibre complex is an important source of beneficial molecules for the host, such as β -glucans, fructans, resistant starch, and arabinoxylans. The bioavailability of these compounds in whole grain products generally depends on different technological issues [54]. It is known that the enrichment of breads with DF requires certain adjustments of several process parameters to obtain a high quality that is acceptable to most consumers; one option for improving it is the use of sourdough biotechnology [55]. When a sourdough process is adopted, the fibres change their chemical and physical properties according to the degree of fermentation [54].

Enzymatic action can change the ratio of insoluble fibres to soluble ones during bread making [56]. In sourdough breads, fibre can undergo two types of enzymatic hydrolysis. When flour is hydrated, several hydrolytic enzymes intrinsic to cereals are activated [57], such as hemicellulase enzymes that degrade hemicelluloses. On the other hand, lactic acid bacteria release enzymes with glycolytic activity [58], which are also capable of acting on the fibre present in the dough. Recently, the effects of *Lactobacillus plantarum* fermentation were reported on β -glucans of barley [59]. Fermentation also decreased the molecular weight of β -glucans, which could have a negative impact on the physiological activities of barley β -glucan, especially in relation to glucose and lipid metabolism.

Different studies have been carried out on bread made using the sourdough technique for its enrichment with dietary fibre. Mihhalevski et al. (2013) [60] evaluated dietary fibre in a rye sourdough bread and concluded that the proportion of both soluble and insoluble dietary fibre increased during rye sourdough processing. They attributed this difference to biochemical and microbiological processes that occur during sourdough bread making. The increase in total dietary fibre content was caused by the formation of resistant starch, and the increase in soluble dietary fibre content can be explained by the conversion of insoluble to soluble fibre during fermentation of rye flour. Such a redistribution of dietary fibre formed by the activities of the intrinsic enzymes in rye flour (amylase, xylosidase,

arabinofuranosidase, b-glucanase, endo-xylanase, and cinnamoyl esterase) was also suggested by Boskov Hansen et al. (2002) [57].

De Angelis et al. (2009) [29] used sourdough to improve the organoleptic quality of breads enriched with fibres and to increase the reduction in the glycaemic index achieved with this enrichment. A sourdough elaboration process, with the appropriate selection of lactic acid bacteria according to the added fibre [29], can be considered one of the tools to make low GI bread. Moreover, the breads' specific volume was higher and the sensory analysis indicated that they were preferred for their acid smell, taste, and aroma.

The possibility of using naked barley as a food product is gaining popularity because of its dietary fibre, especially β -glucans. Pejcz et al. (2017) [61] reported that the fermentation of sourdough breads with barley improved the volume, colour, and sensory properties of breads with this cereal. The fermentation process resulted in a higher concentration of dietary fibre, arabinoxylans, and β -glucans. In another study, sourdoughs made from barley flour without sole husks, or from a mixture of 50 g/100 g barley and wheat flour, were characterized from a microbiological and technological point of view, compared to a single wheat flour from sourdough [62]. Results showed that the use of sourdough can be a strategy to improve the quality of barley bread with higher nutritional value and high DF. Furthermore, despite the lower specific volume and higher density of barley breadcrumbs compared to wheat bread, no significant differences were observed after baking or during the shelf life, thus confirming the possibility of successful exploitation of barley flour in the baking industry.

It has also been shown that the fermentation of sourdough leads to an increased activity of certain enzymes, leading to solubility of arabinoxylans expansion [63], just as sourdough was shown to improve dough and bread structure quality of breads containing whole grains of barley, but little effect on breads prepared with refined barley flour [64]. This positive effect on bread quality could be due to a softening effect on bran particles during fermentation, which would result in less impediment of the gluten network and in the formation of gas cells in the dough [64]. On the other hand, wheat bran fermentation by *Lactobacillus rhamnosus* tripled water extractable arabinoxylans in wheat bran due to endoxylanases activity on high molecular weight arabinoxylans [65,66], which makes these more easily usable by the intestinal microbiota [67]. The soluble dietary fibre (SDF) content was also increased in the fermented wheat bran, which is consistent with the data for SDF in rye sourdough production [60].

Other carbohydrates that have gained interest in recent years are the fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs). The components of the raffinose family oligosaccharides (RFOs) in legume flours, and the fructans in cereal flours, are particularly noteworthy [68]. In general, they are not easily digested carbohydrates that can lead to the development of pathologies, such as irritable bowel syndrome (IBS) or non-celiac gluten sensitivity (NCGS) [69]. They are equally related to osmotic diarrhoea, inflammation, and abdominal distension, and it is known that a reduction in FODMAP content improves the health status of people suffering from any of these pathologies [18].

However, the use of sourdough can contribute to a significant reduction in FODMAPs content without affecting the content of slowly fermented dietary fibre [18]. Therefore, fermented sourdough products are suitable for consumption in low-FODMAP diets and are safe for IBS patients [19,69].

In the last five years, the use of sourdough has gained popularity as a tool to reduce the content of FODMAPs, as well as antinutrients, in grain and cereal products. In this line, Ziegler et al. (2016) [70] indicate that long fermentations of more than 4 h allow for a reduction in FODMAPs, especially fructans, due to their degradation, by up to 90%, in doughs of flours from *Triticum monococcum* and *T. dicoccum*.

3. Influence of Sourdough Fermentation over Proteins

The hydrolysis of the proteins contained in the flours helps, from a health perspective, create products intended to reduce the adverse reactions caused by gluten, or to reduce the risk of

contamination by gluten [8]. In recent years, there has been significant progress in the research on the role of sourdoughs in enabling individuals affected by gluten sensitivity or various gastrointestinal disorders to consume cereal-based products [47].

This is due, among other factors, to the fact that the LAB and yeast of the sourdough synthesize proteases that promote the hydrolysis of gluten [10,71]. Similarly, the reduction in pH facilitates the activation of endogenous enzymes in cereals, as well as the solubilisation and depolymerisation of gluten proteins [72–74]. After fermentation, there is an increase in the presence of protein fragments from the degradation of gliadins and of the high molecular weight subunits of glutenins [75]. It is noteworthy that proteases, both those contributed by microorganisms and those endogenous to the raw materials, influence the volume and texture of products, especially bread [76].

In this sense, there are several strategies to increase protein hydrolysis. Phenomena such as grain germination prior to flour production are positioned as a tool to boost gluten degradation by increasing protease content [77,78]. It may also be beneficial to use flours with high proteolytic activity [79], such as those from brewers' spent grains [80].

It should be noted that if complete degradation of gluten is achieved, the products obtained could be safe for celiac patients [81,82]. With respect to bread, Rizzello et al. (2016) [83] managed to elaborate gluten-free bread, with a high protein digestibility, after the elimination of gluten by the action of sourdough. Some studies, such as Curiel et al. (2014) [84], show that it is possible to reduce gluten to a residual concentration of less than 10 ppm also in products other than bread, such as pasta. However, a total lack of gluten affects the commercial quality of the products [12], as it is difficult to produce breads with adequate volume and texture, and this may have an effect on the consumer acceptability. Therefore, total degradation of the gluten is not always convenient.

Although protein degradation can be an alternative to obtain products suitable for the celiac collective, this technique presents serious disadvantages. On the one hand, most research studies on sourdough do not manage to reach the maximum doses of gluten allowed by the different legislations, or they do not measure it. On the other hand, in the case of managing to reach such maximum, the degradation of gluten would be so extensive that it would be difficult to make bread, where gluten plays an essential role. Then, when taking protein degradation to the commercial practice, an exhaustive control of gluten levels in all batches would be necessary.

Finally, when sourdough is used, the concentration of free amino acids is higher than when only baker's yeast is used [15], although it is possible to combine both. Inoculation with certain LAB strains succeeds in doubling the concentration of certain amino acids, such as leucine, isoleucine, histidine, and lysine, thanks to proteolysis processes [85]. Protein degradation during fermentation that takes place in the sourdough processing also gives rise to potentially health-promoting peptides, such as short branched-chain amino acids and small-sized peptides [86]. These compounds have been shown to contribute to the regulation of insulinemic response, providing protection against type 2 diabetes mellitus and cardiovascular disease [86].

4. Influence of Sourdough Fermentation over Lipids

The presence of lipids in the sourdough is very limited compared to other compounds, such as starch or protein, due to the low lipid content of the flours. This content will be somewhat higher in the case of sourdoughs obtained with whole flours, due to the higher fat content of the germ [87]. Particularly in these cases, it is known that sourdough fermentation reduces fat rancidity by limiting the lipase activity due to the lowering of the pH [88]. This reduction in lipid rancidity in the sourdough contributes positively to the final bread aroma by minimizing unpleasant aromas [89]. The action of the enzymes present in the dough modifies the lipid profile of the flours by partial hydrolysis of the triglycerides and diglycerides. This increases the percentage of monoglycerides and maintains a stable sterol ester content, although the influence on the nutritional characteristics of the bread is limited due to the low level of lipids in the product [90,91]. Considering the reduction of the fat rancidity phenomenon due to the low pH generated, it should be assumed that the hydrolysis of the triglycerides

occurs in the first phases of the development of the sourdough. As already known, monoglycerides are products that help in retarding phenomena as bread staling, as they reduce starch retrogradation, allowing the extending of bread shelf life [92].

As a remarkable aspect, the action of certain microorganisms on some lipids may have other positive aspects. Thus, it has been proven that certain strains of *Lactobacillus hammesii* can convert linoleic acid into monohydroxy octadecenoic acid, which has shown antifungal activity in bread [93]. Therefore, its incorporation in sourdough could increase the shelf life of breads, beyond its beneficial effect by simply lowering the pH.

5. Sourdough Fermentation and Vitamins

The most relevant vitamins in cereal products are vitamin E, thiamine, and folates, which are concentrated in the germ and bran [22,23]. Pseudocereals, in turn, contain vitamin A, as well as vitamin E and folate [94]. To increase the vitamin content, fermentation with sourdough can be applied. Certain strains of LAB can synthesise vitamins such as riboflavin, thiamine, and folate [17,24,95–97]. Mihhalevski et al. (2013) [60] also showed that the content of nicotinamide increased during processing by tenfold, presumably due to microbial activity during sourdough fermentation.

Therefore, the use of LAB in sourdough is well positioned as an alternative to help in the prevention of clinical and subclinical vitamin deficiencies [98]. Strategies such as the selection of vitamin-overproducing strains to increase bioavailability should be applied [99]. In addition, it is presumed that *S. cerevisiae* may stimulate the growth of LAB by producing vitamins not contained in wheat flour such as B12, C, or D [100]. In this regard, Chawla and Nagal (2015) [101] point out that the presence of yeast promotes the formation of folates and thiamine.

Additionally, the use of flour from sprouted grains can improve the availability of the vitamins present in them, since they are generated together with other bioactive compounds when they leave the dormant state [102].

Despite the techniques mentioned above for increasing the concentration and bioavailability of vitamins, the baking process leads to a reduction in the vitamin content. Mihhalevski et al. (2013) [60] establish that the concentration of thiamine, nicotinic acid, pyridoxal, riboflavin, and pyridoxine is reduced by temperature increases. Vitamin B12 is also reduced in the process, both in its added form, cyanocobalamin, in its natural form, hydroxocobalamin, and in its synthesized in situ form [103]. The search for techniques to maintain accessibility and vitamin levels in the products is therefore still necessary. However, the higher the amount of vitamins before baking, the higher the final amount of vitamins after the process.

6. Sourdough Fermentation and Mineral Bioavailability

The most relevant minerals in cereals are K, P, Mg, and Zn [22], but they are deficient in others such as Fe [104]. Certain compounds contained in cereals and grains, especially phytic acid, can reduce the bioavailability of minerals as explained above. It is an acid present in grains and cereals in a variable quantity, and an antinutritional factor that prevents the absorption of Ca, Fe, K, Mg, Mn, and Zn, with which it forms phytates, making them insoluble [10]. Phytates are concentrated in the outer layers of the grain and can be hydrolysed by the phytase present within it [105]. Therefore, the efficiency of wheat bran sourdough fermentation in the hydrolysis of phytates and the solubility of minerals has been studied in comparison with whole wheat flour [106]. It was concluded that the pre-fermentation process of whole grains or bran, under adequate conditions of hydration, allows the degradation of most of the phytic acid and an optimal bioavailability of the minerals. In the fermentation stage, the breakdown of polysaccharides is generally greater with sourdough fermentation than with yeast fermentation [107]. However, this action needs the right pH, time, and temperature conditions, which usually occur during fermentation. The simple fermentation and the phytase activity itself contribute to improve the bioavailability of the minerals, as well as the use of sourdough. Therefore, fermentation with sourdough is well positioned as an effective tool to increase the bioavailability of

minerals. Lopez et al. (2003) [107] point out that it improves bioavailability, mainly of Mg, Fe, and Zn. Other studies note that sourdough fermentation can increase the release of Fe up to eight times [108]. It may even help in the protection against oxidative stress through the biotransformation of Se, thanks to its conversion to a bioaccessible form [109]. The action of strains of the genus *Lactobacillus* spp. manages to increase the bioavailability of Ca, Zn, and Mg [110]. Yeast strains isolated from sourdoughs, such as *Kluyveromyces marxianus*, can also reduce the phytate content [111]. Combination strains of LAB and yeasts lead to reductions in phytic acid content of more than 40% [112]. Regarding the combination of baker's yeast and sourdough, it can increase the concentration of Ca and Mn in the bread, and, in addition, prolonged fermentations lead to an improvement in the solubility of Mg and P due to acidification [113].

In addition, the pH reduction caused by acidification as a consequence of sourdough fermentation increases the activity of endogenous phytase in the grains, making them even more effective than those of microbial origin [114,115]. The resulting action of a combination of endogenous phytases from different cereals can even completely hydrolyse phytic acid [116]. During the fermentation of legume flour, reductions in phytates due to degradation have also been recorded [117,118].

It has been possible to successfully isolate different LAB strains of the genera *Pediococcus* and *Bifidobacterium*, with high phytase activity [119–121] or to increase their phytase expression through their modification [122].

7. The Role of Sourdough Fermentation on the Levels and the Stability of Bioactive Compounds

Fermentation enhances the presence of bioactive compounds which allow the prevention of various pathologies and diseases related to metabolic syndrome or to cancer [123]. As for cereals, the phytochemicals are divided into flavonoids and non-flavonoid phenols, the latter being more abundant [16]. Since they are mainly found in the outer layers of grains, wholegrain flours are rich in phytochemicals. However, these compounds are sensitive to air contact and their level is reduced in baking processes [23].

The presence of phytochemicals, with antioxidant activity potentially beneficial to health, such as free phenolic acids, total phenolic compounds, or alkylresorcinols, increases with the reduction in pH caused by fermentation processes [124]. Sourdough fermentation contributes to a significant increase in bioactive compound levels and antioxidant activity [123,125–127].

A number of LAB strains are capable of synthesising peptides with an ex vivo antioxidant activity, and with anti-inflammatory and free radical scavenging activities during the fermentation of cereal flours [128,129]. These peptides also maintain their activity in the final product [130]. Colosimo et al. (2020) [131] estimate that fermentations of 72 to 96 h achieve optimal antioxidant activity by increasing the presence of amino acids, organic acids, and aromatic compounds with antioxidant potential. The amount of these compounds is also increased by the protein hydrolysis caused by microbial activity. It should be noted that wheat flour harvested at an advanced stage of maturation presents a greater quantity of bioactive compounds [132], which can reduce the previously mentioned fermentation times.

Meanwhile, other studies have pointed out that the antioxidant activity depends on the type of inoculum and the substrate used in the fermentation [133,134], with numerous examples found in the literature. In the case of *Pediococcus acidilactici* strains incorporated to barley sourdough, they can increase the content of phenolic compounds by 34.6% and the activity of free radical scavengers by 79.7% [135]. The fermentation of durum wheat grain and khamut flour with *L. sanfranciscensis* and *L. brevis* strains slightly increases the release of flavonoids [54]. Lactic acid fermentation also acts in the delivery of bioactive peptides from legume proteins and with the biotransformation of phenols into compounds with higher activity [118]. Strains of *L. acidophilus* that can produce exopolysaccharides (EPS) with antioxidant potential [136] have also been identified. The use of probiotic microorganisms, such as *Enterococcus faecium* and *Kluyveromyces aestuarii*, is currently being studied to boost the antioxidant capacity of bread by increasing the concentration of phenolic compounds [111]. Brewer's spent grain

might also be an option to consider since the enzymatic activity it has been subjected to increases its antioxidant potential [137].

In the sourdough, due to the hydrolysis of the proteins, low molecular weight peptides with angiotensin-converting enzyme (ACE) inhibitory activity are also released [138,139]. These peptides with antihypertensive effects are mainly found in wholemeal flours [140]. Research suggests that, despite the reduction in the content of these peptides during heat treatment, their levels in bread are at a concentration required for their activity in vivo [141]. Fermentation with certain strains of the genus *Lactobacillus* spp. can contribute to increase the concentration of this peptide up to seven times [142].

Moreover, γ -aminobutyric acid (GABA) is a non-protein amino acid, acts as a neurotransmitter, has hypotensive, diuretic, and tranquilizing effects, derived from the decarboxylation of glutamate mediated by enzymes, and it is present in the grain or is generated by some LAB [88]. A remarkable fact is that breads fermented with sourdough have higher levels of GABA than other commercial breads [139,143]. Certain *L. plantarum* and *L. lactis* strains can increase the concentration of GABA in cereal, pseudocereal, and legume flours [144]. Curiel et al. (2015) [117] also detected significant increases in legume flours after sourdough fermentation.

Among the bioactive compounds that see their concentration incremented with fermentation, lunasin stands out. *L. curvatus* and *L. brevis* strains detected in sourdough fermentation can synthesize this compound, a cancer-preventing peptide, increasing its concentration two to four times during fermentation [145]. Studies on legume flour have identified that lunasin-like polypeptides' concentration rose during fermentation due to proteolysis processes [146]. *L. plantarum* and *L. rossiae* strains have also shown a potential for the release of biologically active benzoquinones, which have exhibited antitumour activity in ex vivo trials [147].

Lastly, during the fermentation of buckwheat flour, it has been observed that compounds with antioxidant activity, such as quercetin, remain both in the dough and in the final product [148]. By fermenting rye bran, the availability of phytochemicals is enhanced by increasing the presence of free phenolic acids, released by the degradation of benzoxazinoids and alkylresorcinols [149].

8. Sourdough and Regulation of Gut Microbiota

The term microbiome refers to the total number of microbes and their genetic material, while the microbiota is the microbial population present in different parts of the human body [150]. These microbial populations explain critical features of human biology and play an important role in the regulation of human health and disease. This microbiota is able to degrade and ferment complex carbohydrates in the large intestine to produce basic energy recovery and regulate the health of the intestine through the metabolites produced [151]. The diet and with it, the intake of specific polysaccharides, can change the composition and metabolic activities of the intestinal microbiota, promoting the growth of healthy microbes (*Bifidobacterium*, *Lactobacillus*, or butyrate producing bacteria), the production of short chain fatty acids (SCFA), and the reduction in pH, inhibiting effects towards pathogens [67]. They thus affect the health of the host and the gut by modulating inflammation, and glucose and lipid metabolisms [151]. Fermented products in general and cereal-based foods in particular are characterized by an abundance of ingredients that reach the gastrointestinal tract and are accessible by the gut microorganisms of the host [152]. It is known that sourdough fermentation has the ability to actively delay the digestibility of starch, which can increase the production of indigestible polysaccharides that escape from the small intestine, along with the grain fibres, and can then be fermented by the colonic microbiota [33]. The fermentation of the sourdough can influence intestinal health by different mechanisms, such as modulating the dietary fibre complex and its subsequent fermentation pattern, producing exopolysaccharides (EPS) with prebiotic properties, and/or providing metabolites from LAB fermentation that influence the intestinal microbiota [23]. Certain LAB produce exopolysaccharides, such as glucan, fructans, and gluco- and fructo-oligosaccharides that have potential gut health-promoting properties. Intestinal microbes can metabolize these compounds that have been shown to possess prebiotic properties [153–155]. In this way, the metabolism of these compounds can

generate propionic acid, which has several beneficial effects [156], such as reducing cholesterol and triglyceride levels, and increasing insulin sensitivity.

Although research suggests that the consumption of sourdough breads may be especially beneficial for the modulation and maintenance of the human intestinal microbiome profile, studies on how the consumption of sourdough breads may have effects on both the intestinal microbiota and the release of health-promoting metabolites, are scarce. In this regard, Abbondio et al. (2019) [157] evaluated how these effects influence the composition and function of intestinal microbiota by feeding rats a diet supplemented with sourdough breads. As a result, supplementation of the diet with sourdough bread led to a reduction in specific members of the intestinal microbiota associated with low-protein diets or known intestinal pathogens, and *Bacteroides* spp. and *Clostridium* spp. were detected in larger quantities. In addition, recent research interestingly suggests that the cell wall components of *Lactobacillus plantarum*, a strain present in the sourdough, have the ability to stimulate the immune response in the intestine, even when the bacteria are not alive [158].

Another important contribution of sourdough to the health of the human intestine and the regulation of its microbiome is that the bacterial strains isolated from it have shown to be potential probiotics [159–161]. However, alternatives are still being studied that may respond to the fact that probiotic bacteria present in the sourdough die during the baking process. Although it is undeniable that the process of sourdough fermentation results in a compound-rich product shown to promote human health benefits, it is important to study in more detail the specific effect of sourdough on the intestinal microbiome.

9. Future Trends

Advances in understanding the capacity of improving health-related properties with sourdough fermentation are explained by the extensive research carried out in the last 15 years. Figure 2 provides an overview of the potential of this technology for grain processing.

More than 10 years ago, the reviews by Katina et al. (2005) [22] and Poutanen et al. (2009) [23] ventured a series of future trends, most notably the following: (1) regulation of microbiota, (2) development of products with probiotic properties, (3) optimization of processing conditions, (4) improvement of products other than bread, and (5) increased concentration of bioactive compounds and microbial metabolites.

The last three points are those where most progress has been made [3,10,29,84,102]. Points 3 and 4 are not specific to this review and, although much research has been conducted in recent years, there are still aspects to be studied about point 5, such as the incorporation of certain ingredients rich in fibre, highly digestible starches, or proteins in these microbial metabolites. In addition, there is a greater need for an in-depth regulation of the microbiota and the way to obtain products with probiotic potential, especially because of their close relation with a potential improvement in health. On the other hand, the optimization of the process must focus on a balance between healthy properties and sensory quality.

Many of the trends mentioned are still under study, especially the increase in the concentration of bioactive compounds and microbial metabolites. However, there are other future trends that are currently emerging and have not been addressed by previous reviews. Sourdough fermentation can also help fight inflammatory and high oxidative stress processes among consumers in developing countries [162]. Sourdough fermentation is therefore positioned as a useful tool for health promotion in societies where access to healthcare is not widely spread.

Another trend for the future would be the use of sourdough to reduce the content of harmful components in grain and cereal products, although the literature on this phenomenon is not yet extensive. Nevertheless, it has already been shown that sourdoughs may help reduce the content of sugar added to products by the presence of polyol-producing LAB [163]. They can also prevent the development of pathogens, such as *Clostridium*, by generating conditions that induce states of metabolic latency in them [157]. In addition, some LAB isolated from sourdoughs are able to prevent the growth

of mould [164] and have an anti-aflatoxigenic effect [165]. Finally, the reduction in acrylamide, another compound of major concern in grain and cereal products, by sourdough fermentation, has been addressed, for instance, by Bartkiene et al., 2017 [166]. For future research, it would be interesting to expand knowledge about the microbiota, the temperature, or the raw materials employed for obtaining sourdough.

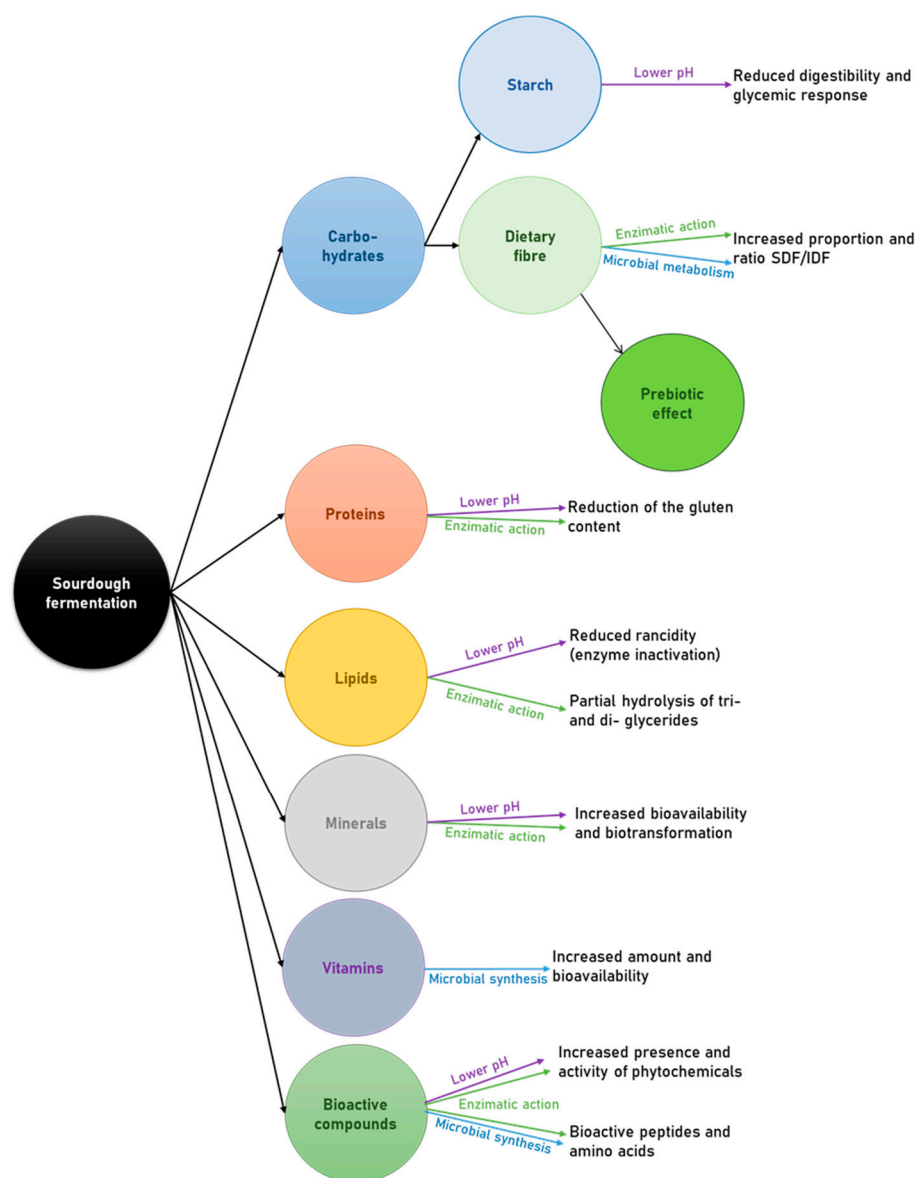


Figure 2. Potential of sourdough fermentation to modify the nutritional quality of cereal and grain-based products.

10. Conclusions

Research on sourdough conducted before the period under study here focused on technological improvements and on the reduction in the gluten content and the glycaemic index of breads. In the last 15 years, although these aspects have remained under study, new research has been initiated on the use of sourdough to improve the potential of grain as well as cereal products and thus, enhance health through various modifications. Improvements cover the modification of nutrients quantity and bioavailability, the generation of bioactive compounds, as well as the reduction of compound contents which could be harmful to certain segments of the population.

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