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Thirty years of knowledge on sourdough fermentation: A systematic review

Kashika Arora^{a,1}, Hana Ameer^{a,1}, Andrea Polo^a, Raffaella Di Cagno^a, Carlo Giuseppe Rizzello^b, Marco Gobbetti^{a,*}

^a Faculty of Science and Technology, Libera Università di Bolzano, Piazza Università, 5, 39100, Bolzano, Italy

^b Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Via G. Amendola, 165/a, 70126, Bari, Italy

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ABSTRACT

Background: Sourdough is one of the oldest examples of natural starters, mostly used for making fermented baked goods as an alternative to baker's yeast and chemical leavening. Almost 30 years of research have accumulated showing its performance. Time is mature to elaborate collectively these data and to draw conclusions, which would represent milestones for scientists, industries and consumers.

Scope and approach: With the scope of highlighting its microbiological, biochemical, technological and nutritional potential, we used "sourdough" as the only keyword and the PRISMA flow diagram to retrieve, select and systematically review 1230 peer reviewed research articles from four databases (Google Scholar, Scopus, PubMed and ScienceDirect).

Key findings and conclusions: The literature states that sourdough baked goods underwent characterization in almost 50 countries and all continents, mainly dealing with salty (breads and substitutes) and sweet products. Converging data defined optimal use conditions, most common microbiological and biochemical characteristics, criteria for selecting and re-using starters, and versatility of sourdough for making baked goods with a relevant number of flour species/varieties and agro-food by-products. Because of the unique microbial composition and functionality, sourdough has claimed as an irreplaceable starter for improving the sensory, rheology and shelf life attributes of baked goods. The most recent literature showed how the sourdough fermentation mainly increased mineral bioavailability, enabled fortification with dietary fibers, lowered glycemic index, improved protein digestibility and decreased the content of anti-nutritional factors. This knowledge is solid for delivering to industries and consumers, and to face new research challenges starting from a consolidated state of the art.

1. Introduction

A widely accepted technical definition describes the sourdough as a mixture of flour and water, spontaneously fermented by lactic acid bacteria and yeasts, and having acidification and leavening capacities (Gobbetti, 1998). Sourdough is one of the oldest examples of natural starters, mostly used for making leavened baked goods as an alternative to baker's yeast and chemical leavening.

Leavening/fermentation and bread have always been central elements of the history, whose narration reflects the human spirituality and civilization. Probably, Egypt is the motherland of sourdough bread (Guidotti, 2005). In the Egyptian dialect, the pronunciation of ferment and bread uses the same Arabic term *aish*, which means life. Since centuries, the Catholic religion magnifies leavening and bread with meanings and metaphors; one of the most representative is "food of

eternal life". In the *Tacuina Sanitatis* (11th century), the bread becomes the key element of many religious reproductions, already assuming a nutritional meaning: "white bread improves human wellness, but it had to be completely fermented". To date baked goods and, in particular, leavened bread are fundamental foods for planet sustenance. UNESCO includes the bread within the list of the intangible heritage of humanity. The leavened/fermented bread is a basic component of the Mediterranean diet, somewhat representing the modern projection of the Benedictine monastic diet (Archetti, 2014).

Despite this historical traceability and the central role of leavened baked goods in almost all dietary habits, the sourdough fermentation attracted the scientific attention not more than 30 years ago. Spicher and the Spanish group coordinated by Benedito de Barber were the first who consistently studied the sourdough's world (e.g., Barber, Torner, Martínez-Anaya, & de Barber, 1989; Spicher, 1987). The first landmark

* Corresponding author.

E-mail address: Marco.Gobbetti@unibz.it (M. Gobbetti).

¹ These authors equally contributed to this work.

description of *Lactobacillus sanfranciscensis* (*Fructilactobacillus sanfranciscensis*) appeared in 1971 (Sugihara, Kline, & Miller, 1971). Compared to researches dealing with other fermented foods and beverages (e.g., cheeses, yogurt, sausages and wine), the temporal delay was mainly because the almost unique use of baker's yeasts, which accompanied the industrial and artisanal manufacture of baked goods until late 1900s. Since approximately 1990, a systematic research activity begun aiming at rediscovering the potential of sourdough fermentation and allowing the inevitable technology transfer. The initial focus was on the technological effects of sourdough fermentation with respect to flavor, rheology and shelf life (e.g., delay of staling, spoilage prevention), and on the microbial interactions in such complex ecosystem. Concomitantly, the abundant microbial diversity and, in particular, the succession of dominating and sub-dominating populations of lactic acid bacteria promoted studies on the sourdough assembly and composition. The house microbiota, type of flour, additional ingredients and tap water were the main microbial sources and/or drivers to establish the potential of this natural starter. More recently, the focus shifted towards the multiple nutritional advantages offered by sourdough fermentation with respect to the other leavening agents. A number of reviews succeeded focusing specific aspects (see Corsetti & Settanni, 2007; De Vuyst, Van Kerrebroeck, & Leroy, 2017; Gänzle, Loponen, & Gobbetti, 2008; Gobbetti, 1998; Katina, 2005; Minervini, De Angelis, Di Cagno, & Gobbetti, 2014), but none systematically reviewed the overall literature and no comprehensive data are yet available on technological and nutritional issues, which unequivocally claim the sourdough potential.

Here, we systematically reviewed the sourdough literature since the last 30 years with the aim of reporting and definitively highlighting the microbial diversity, and the technological and nutritional potential of this natural starter. We believe that we have now sufficient literature data to solidify this fundamental step forward for microbiologists, technologists, nutritionists, food science and industry and for society as a whole.

2. Literature search methodology

The timeline for our literature survey was set to the last 30 years (January 1990–February 2020), using only “sourdough” as keyword. Additional literature with respect to this keyword was only used for limited and specific purposes (flour microbiota and other ingredients). Peer reviewed research articles sourced from four databases: Google Scholar, Scopus, PubMed and ScienceDirect. The PRISMA flow diagram (Supplementary Fig. 1) shows the screening criteria and lists the number of research articles considered in our systematic review. Initially, “sourdough” as search keyword from all databases resulted in 3116 research articles. The elimination of duplicates reduced the number to 1468. The screening strategy included full text research articles only in English language, and excluded reviews, book chapters, thesis dissertations and conference proceedings. Applying our screening criteria, the final number was 1230. The supplementary Dataset lists all the literature references used in this review, including those reported in the Reference section. We know that some authors, even using sourdough, currently refer to flour lactic acid fermentation, especially when the focus is on selected starters. Obviously, these few research articles remained out from our Dataset because we made the choice to refer strictly to sourdough.

Starting from 1990 and grouping sourdough research articles every five years, except for 2020 because incomplete, the number increased, respectively, from 33, 56, 130, 251, 372 to 582, which gives an order of magnitude of the growing interest (Supplementary Fig. 2).

3. Baked goods and flours

A relevant number of research articles (280) dealt with the microbiological, biochemical and/or technological features of typical/traditional sourdough baked goods, which spread in 47 countries and all

continents (Fig. 1). The major part (246) characterized salty products (breads and substitutes), the remaining (24) dealt with sweet baked goods or both the categories (10). In Europe, Italy was the leading country with the characterization of more than 30 traditional varieties of salty and sweet sourdough baked goods. Emblematic reports (Lattanzi et al., 2013; Minervini et al., 2012) showed the distinguishing compositional and functional features of sourdoughs used for making 19 typical breads and 18 sweet baked goods. Almost the same approach distinguished sourdoughs used for traditional French breads (baguettes), brioche and rolls (e.g., Lhomme et al., 2015). Remaining in Europe, studies from Germany, Belgium, Scandinavia and the Baltic area mainly deepened the sourdough rye bread tradition (e.g., Ua-Arak, Jakob, & Vogel, 2017). In Asian countries, Iranian Barbari, Chinese steamed and Indian Bhatara sourdough breads underwent investigation (e.g., Zhang, Zhang, Sadiq, Arbab, & He, 2019). In Africa, Egyptian Balady, Sudanese Kisra and Ethiopian Injera sourdough breads were some of the most popular (e.g., Baye, Mouquet-Rivier, Icard-Vernière, Rochette, & Guyot, 2013). The main sourdough products in South America were Mexican Tortillas, while industrial sourdough breads, rolls, crackers and cookies attracted the interest in United States. Almost all the 280 articles concluded on the uniqueness of the microbial composition and functionality of each sourdough for every baked good. With an increasing trend, some recent research articles (16) dealt with the use of sourdough also for making pasta. The sourdough fermentation affected both sensory and rheology attributes.

The proof of the irreplaceable sourdough potential comes from the extraordinary number of flours and agro-food by-products successfully fermented (Supplementary Fig. 3). Apart from the variable processing, the common purpose of these research articles was to exploit the sourdough potential for increasing the technological and nutritional attributes of conventional and non-conventional flours, and to recycle agro-food by-products. In detail, flours from 23 species/varieties of cereals, also using sprouted seeds, 10 pseudo-cereals, 19 varieties of legumes and 25 miscellaneous vegetables were suitable for sourdough fermentation. While the research activity in the interval 1990–1999 mainly dealt with soft and durum wheat and rye, the most recent research articles enlarged the spectrum of cereal flours and mainly concerned legumes and pseudo-cereals, also used for gluten-free formulations, and other vegetable matrices. This trend found a consolidation starting from 2007. In particular, the sourdough was the natural starter to ferment 19 Italian varieties of legume flours, which, after fermentation, were suitable for using alone or better in combination with cereal flours (Curiel et al., 2015). Sourdough fermentation of legume flours increased the contents of free amino acids (FAA), γ -amino butyric acid (GABA), polyphenols, dietary fibers (DF) and bioavailable minerals, promoted antioxidant activities and the *in vitro* protein digestibility, and lowered the glycemic index (GI) (Coda, Rizzello, & Gobbetti, 2010; Gabriele et al., 2019; Rizzello, Calasso, Campanella, De Angelis, & Gobbetti, 2014). Starting approximately from the last decade, several research articles (30) demonstrated how the sourdough fermentation was the unique tool for improving the rheology, sensory, shelf life and nutritional attributes of gluten-free formulations made of mixtures of rice, corn and several pseudo-cereals. Numerous and heterogeneous agro-food by-products were recyclable by sourdough fermentation, almost all milling by-products and a diversity of other miscellaneous agricultural wastes. In practice, the sourdough fermentation was the irreplaceable technique for getting a consistent increase of the bran content in various baked good formulations (Pontonio et al., 2020). At the same time, the sourdough fermentation had the potential to inhibit the lipase activity of the cereal germ, which allowed a prolonged shelf life and its use as nutrient-rich ingredient in bread making formulas (Rizzello, Nionelli, Coda, De Angelis, & Gobbetti, 2010).

4. Using conditions

Once prepared, the use of mature sourdough depends on the



Fig. 1. Worldwide map of sourdough products (salty and sweet). Countries with sourdough products subjected to characterization are in dark gray, and the total number of research articles in each country is within brackets.

tradition and type of baked goods. A scientifically accepted classification of sourdough categorizes three types. Type I with almost daily back slopping to keep the microorganisms in an active metabolic state; type II with propagation at relatively high temperatures (>30 °C) and long fermentation time (up to 5 days), mainly acting as acidifying and aroma carrier; and type III corresponding to the dried sourdough used as flavoring agent (De Vuyst & Neysens, 2005). The 272 research articles consulted for this paragraph all referred to type I sourdough, although the other two types recently achieved some industrial relevance. A variable number (2–10) of back slopping (refreshments) may precede the final sourdough fermentation before baking but the most common practice is the one-step process. The median time used for sourdough fermentation is 4 h, with extreme values of 1–8 h, which, respectively, correspond to the mixed use with baker's yeast or to long-time traditional protocols for making specific baked goods (Fig. 2A). Usually, the time of fermentation is set for achieving suitable acidification, leavening power and cell densities of lactic acid bacteria and yeasts. Prolonged time of fermentation (up to 24 h) accompanied the sourdough fermentation of agro-food by-products (e.g. wheat germ, brewer's spent grains) to render them suitable as ingredients for bread making (Rizzello et al., 2010). The most common temperature used for sourdough fermentation

is 30 °C (Fig. 2A), with variations that range from 22 to 40 °C, which undoubtedly affect the overall biochemical and sensory characteristics (Vrancken, Rimaux, Weckx, Leroy, & De Vuyst, 2011). Commonly, the percentage of sourdough inoculum varies from 10 to 25%, some outlier procedures also consider percentages less than 5 or up to 50% (Fig. 2B). The median value is 20%. The percentage of sourdough inoculum has proven to influence not only the fermentation rate but also the synthesis of exopolysaccharides (EPS) (Kaditzky & Vogel, 2008), vitamin content (Batifoulie, Verry, Chanliaud, Rémésy, & Demigné, 2005), and sensory and rheology attributes (Katina, Heiniö, Autio, & Poutanen, 2006). Regardless of the protocol used, the median cell densities found after sourdough fermentation are log 8.5 and 6.5 CFU/g for lactic acid bacteria and yeasts, respectively (Fig. 2B). These values allow an estimated ratio of 100:1. For several sourdoughs, it narrows to 10:1. Usually, these two ratios underlie the optimal sourdough performance as long as the cell densities of lactic acid bacteria and yeasts are not below log 8.0 and 6.0 CFU/g, respectively. Exceptionally, lactic acid bacteria are detectable below log 3.0 CFU/g or above log 9.0 CFU/g, which are, respectively, the cases of using commercial dried sourdoughs (Principato, Garrido, Massari, Dordoni, & Spigno, 2019) or starter cultures directly added to the dough (Pontonio et al., 2015). The same variations almost

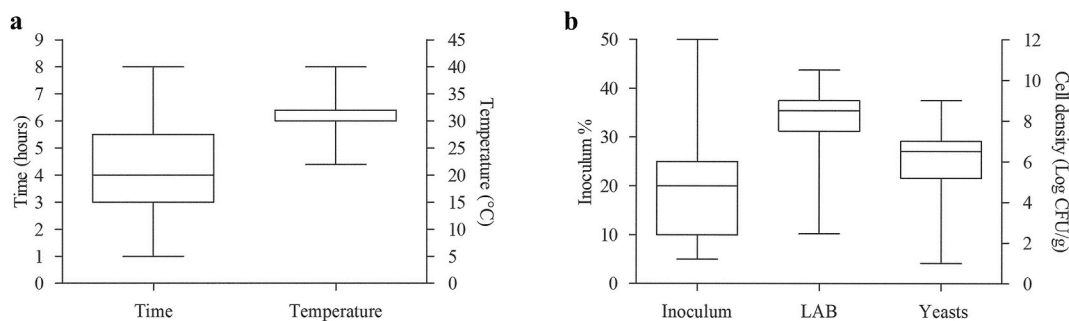


Fig. 2. Box plots showing the time and temperature (A), and percentage of inoculum and final cell densities of lactic acid bacteria (LAB) and yeasts (B), which characterize the sourdough fermentation.

5. Microbiological and biochemical characteristics

We found 312 research articles that, in most of the cases, combined the microbiological and biochemical characterization of sourdoughs. Regarding the microbiological characterization, 233 research articles used culture-dependent methods, 29 dealt with culture-independent techniques, and 50 used both the approaches (Supplementary Fig. 4). The pioneer study for culture-independent methods was by Ehrmann, Ludwig, and Schleifer (1994) who developed a technique based on reverse dot blot assay, which allowed the direct identification of lactic acid bacteria without cultivation (Ehrmann et al., 1994). Following approaches included PCR-DGGE (Gatto & Torriani, 2004), direct extraction of total microbial DNA from sourdough (Settanni, Massitti, Van Sinderen, & Corsetti, 2005) and TTGE (Ferchichi, Valcheva, Prévost, Onno, & Dousset, 2007), but only from 2010 onwards culture-independent methods, mainly based on high throughput sequencing, became common approaches to study the sourdough microbial assembly and diversity. To date, no studies are yet available using meta-genome approaches and fundamental research should certainly move in this direction to better deepen the sourdough microbiota assembly and functionality. The main secret for sourdough performance lies in microbial diversity. Exceptionally, sourdoughs harbor few species. In most of the cases, complex microbial consortia colonized this ecosystem. Up to 59 bacterial genera were detectable in sourdoughs: 10 belonging to lactic acid bacteria and 49 to other bacteria (Supplementary Table 1). Most of these other bacteria behave within the community as satellite members (sub-dominant populations), whose eco-physiological role is worthwhile to deepen. The genus *Lactobacillus* is largely the most abundant in sourdough, with 82 species being variously detectable (Fig. 3A). Based on the recent revision of the taxonomy for the genus *Lactobacillus* (Zheng et al., 2020), which also includes sourdough species, we reported also the new names in correspondence of the first citation throughout text or figures. Sixteen species were identifiable in more than 15 worldwide sourdoughs, meaning that they are the most common representatives of this community. *Lactobacillus plantarum* (*Lactiplantibacillus plantarum*) reported in 142 research articles, *Lactobacillus brevis* (*Levilactobacillus brevis*) (93), *L. sanfranciscensis* (90) and *Lactobacillus fermentum* (*Limosilactobacillus fermentum*) (56) were the most common isolates, showing how nomadic species (*L. plantarum*) or hetero-fermentative species dominated. As shown in Fig. 3B, 80 species of yeasts were identifiable worldwide in sourdoughs. They mainly belong to *Saccharomyces*, *Candida*, *Kazachstania*, *Torulopsis*, *Yarrowia* and *Pichia* genera. Although stable associations with lactic acid bacteria for making sweet baked goods also comprised *Kazachstania exigua* or *Kazachstania humilis*, the species usually identified is *Saccharomyces cerevisiae*. The current debate regards the existence of *S. cerevisiae* wild strains or the environmental cross contamination because of the concomitant use of baker's yeast.

Notwithstanding the remarkable role of the house microbiota and additional ingredients, several lactobacilli are described as resilient and resistant in flours and grains, which explain and mirror partly the same dominant microbiota identifiable in sourdoughs. Indeed, competitive *Lactobacillus* species already present in the rye flour (e.g., *L. sanfranciscensis* and *L. fermentum*) became dominant during sourdough fermentation (Meroth, Walter, Hertel, Brandt, & Hammes, 2003). Durum wheat autochthonous *Enterococcus faecium* and *Pediococcus pentosaceus* rapidly acidified the dough, making the ecosystem suitable for sourdough maturation by other *Lactobacillus* species (Corsetti et al., 2007). The large microbial diversity affecting organic wheat, spelt and rye flours shaped the subsequent composition of the sourdough microbiota (Stanzer, Kazazić, Ivanuša, Mrvčić, & HanousekČiča, 2017). The variable microbial dynamics of several spelt sourdoughs reflected the flour autochthonous microbiota, which, in turn, depended on flour origin, cultivation practices and storage conditions (Korcari, Ricci, Quattrini, & Fortina, 2020). Assessing the microbial dynamic of wheat grains after harvesting and during storage, *E. faecium*, *Enterococcus*

durans, *L. brevis*, *Lactobacillus pentosus* (*Lactiplantibacillus pentosus*) and *Lactobacillus paracasei* (*Lacticaseibacillus paracasei*) demonstrated a remarkable capability to overcome these stressing conditions (Gaglio, Cirlincione, et al., 2020).

Some biochemical parameters, relatively simple to determine, commonly describe the sourdough performance. With differences related to type of flour and protocol used, the median value for pH is 4.1, with the most common range between 3.4 and 4.9 (Fig. 4A). Extremely low values of pH (≤ 3.0) were only detectable using particular ingredients (e.g., brewer's spent grains) and long-time fermentation (48 h) (Waters, Jacob, Titze, Arendt, & Zannini, 2012). Combined with the above values of pH, the most common interval for total titratable acidity (TTA) is 4.0–25.0 ml of 0.1 M NaOH/10 g of dough, with a median value of 11.0 ml of 0.1 M NaOH/10 g of dough. Extremely high values of TTA

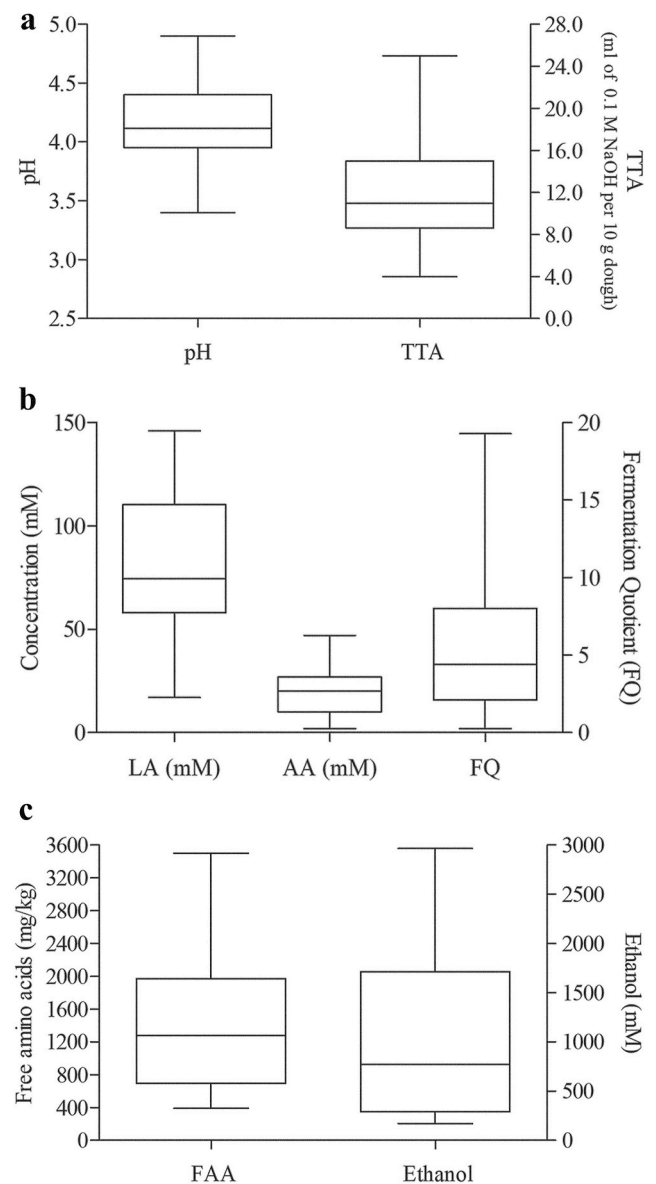


Fig. 4. Box plots showing the range of some biochemical parameters used to characterize the sourdough fermentation. (A) pH and total titratable acidity (TTA; ml 0.1 M NaOH/10 g of dough); (B) concentration (mM) of lactic (LA) and acetic (AA) acids, and fermentation quotient (FQ); (C) concentration of total free amino acids (FAA) (mg/kg) and ethanol (mM). Median values are represented (—) in the plots. The top and the bottom of the box represent the 75th and 25th percentile of the data, respectively. The top and the bottom of the bars represent the 5th and the 95th percentile of the data, respectively.

(40.1 ml of 0.1 M NaOH/10 g of dough) were observable during long time (seven days) hemp sourdough fermentation (Nionelli et al., 2018). Although TTA gives some indirect information on the ratio between lactic and acetic acids, their direct determination is also quite common. The concentration of lactic acid ranges from 15 to 150 mM, with a median value of 75 mM, while that of acetic acid is within 1–50 mM, with a median value of 20 mM (Fig. 4B). The general belief is that higher the level of acetic acid, better the corresponding flavor of sourdough. Therefore, interventions to favor the acetate kinase pathway (e.g., use of external electron acceptors and sugar pentoses, and other metabolisms able to re-oxidize NADH) became routine in sourdough processing (De Vuyst et al., 2002; Gobbetti, Lavermicocca, Minervini, De Angelis, & Corsetti, 2000; Korakli, Rossmann, Gänzle, & Vogel, 2001). The most recommended quotient of fermentation (QF, molar ratio between lactic and acetic acids) is below 5.0. Although with large fluctuations from 0.25 to 20, the elaboration of literature data allowed the calculation of a median value of 4.4. As products deriving from primary and secondary proteolysis through the activities of flour and microbial enzymes, the concentration of total FAA is another biochemical indicator. Because of the marked influence by type of flour, parameters of fermentations and strains used the concentration of FAA after sourdough fermentation largely varies from 390 to 5000 mg/kg, with a median value of 1360 mg/kg. A consistent increase with respect to the initial flour concentration would guarantee the accumulation of enough FAA acting as flavor precursors. As mainly synthesized through the alcoholic fermentation, the median value for ethanol concentration is 771 mM, with much lower levels in baked goods because of the evaporation during baking.

An important step forward in both fundamental and applied researches should allow, as already done for other food and beverage processes, the development of automatized equipment, which permit a rapid and simple monitoring of the main sourdough biochemical performance, so that, even in the smallest industrial plants, a sufficient autonomy in the management of this natural starter would be possible.

6. Use of starters and criteria for selection

The use of newly prepared sourdoughs with selected lactic acid bacteria and yeasts became a common practice for increasing the performance and/or for targeting specific attributes. We retrieved 124 research articles dealing with this practice. Some (31) used autochthonous bacterial isolates but the major part transferred strains from other food ecosystems. All including, the selection considered a quite large spectrum of genera (e.g., *Bifidobacterium*, *Enterococcus*, *Lactococcus*, *Leuconostoc* and *Pediococcus*) but inevitably the major part of strains was from *Lactobacillus*. In particular, strains of *L. plantarum* (73 research articles), *L. brevis* (34) and *L. sanfranciscensis* (31), which agrees with the dominance of these species within the sourdough microbiota (see paragraph 5). Recently, also non-conventional starters from *Leuconostoc* and *Weissella* genera showed interesting capability of adaptation and performances (Montemurro et al., 2020). The combination of multiple selected strains is the most common practice with the aim of reproducing the natural sourdough fermentation. For autochthonous strains, the usual procedure concerns the isolation from flours or traditional sourdoughs, selection, propagation using several back slopping and re-use in the form of selected sourdough. The main sources for isolation, which almost coincided with the matrices subjected to sourdough fermentation, were cereals (mainly soft wheat), pseudo-cereals, legumes and milling by-products (e.g., wheat germ) (Supplementary Fig. 5). Selection criteria are the most diverse, including technological, biochemical and nutritional attributes. Nevertheless, acidification and growth rates are the most screened performances, trying to speed up the sourdough fermentation for making it suitable at artisanal and, especially, at industrial levels. Other largely used criteria consider antifungal activity, EPS formation, synthesis of volatile components, and proteolysis. Focusing on nutritional attributes, synthesis of GABA (Coda et al.,

2010) and angiotensin I-converting enzyme (ACE) inhibitory and antioxidant peptides (Coda, Rizzello, Pinto, & Gobbetti, 2011; Rizzello, Cassone, Di Cagno, & Gobbetti, 2008), degradation of phytic acid (Lopez et al., 2000) and acrylamide (Bartkiene et al., 2017), and digestibility (Mamhoud et al., 2016) are those criteria mostly assessed for selection.

Although with less abundant research articles (63), the selection also concerned yeasts. *Saccharomyces*, *Candida* and *Kazachstania* were the most targeted genera, with the highest number of research articles dealing with *S. cerevisiae*.

Undoubtedly, a wider diffusion of ready-to-use starters will contribute to the wider diffusion of sourdough and to the manufacture on a larger scale of related baked goods.

7. Rheology, sensory and shelf life attributes

An abundant literature dealt with rheology (323 research articles), sensory (227) and shelf life (152) attributes of sourdough fermentation. Several research articles focused on more than one of these attributes and the common aim was to show how the sourdough behaved with respect to baker's yeast. Temporarily speaking, most of the literature on rheology and sensory attributes is retrievable in the period from 2005 to 2015, almost converging on the convenient use of sourdough.

7.1. Rheology attributes

Compared to baker's yeast, the sourdough fermentation improved the rheology attributes of bread, Panettone, flat bread (Piadina), bread rolls, toast bread, burger buns, pizza, biscuits, cakes, crackers and puff pastry. The improvement targeted various attributes, mainly regarding texture (hardness, adhesiveness, resilience, cohesiveness, chewiness, springiness and gumminess), shape, specific volume, crust and crumb color, moisture retention, and crumb structure. Pioneer research articles (Corsetti, Gobbetti, Rossi, & Damiani, 1998; Crowley, Schober, Clarke, & Arendt, 2002) undoubtedly showed the increased bread specific volume and the reduced crumb firmness over time. These superior attributes mainly relied on physicochemical changes of the protein network, which facilitated the larger dough expansion during fermentation (Clarke, Schober, & Arendt, 2002). Slice profiles generated from digital image analysis showed that typically sourdough breads had higher numbers of smaller halos than breads leavened with baker's yeast. Usually, crumb holes of relatively small size (1–2 mm) are desirable, while large and irregularly distributed voids are unpleasant. These effects on rheology were also strain dependent, and *Lactobacillus amylovorus* (Ryan et al., 2011), *L. plantarum* (Moore, Dal Bello, & Arendt, 2008), *L. brevis* (Nami, Gharekhani, Aalami, & Hejazi, 2019) and *Leuconostoc citreum* (Coda et al., 2018), among the others, showed appreciated performance. Other research articles (Chen, Levy, & Gänzle, 2016; Katina et al., 2009) proved the beneficial effects of EPS-producing strains in terms of specific volume and firmness. An abundant literature (125 research articles) was also dealing with the rheology of baked goods made with non-conventional flours (legumes and pseudo-cereals) and milling by-products (bran and germ). For instance, the use of a legume sourdough, consisting of chickpea, lentil and bean flours (15% wheat replacement), allowed the manufacture of bread with higher specific volume than the control bread made with the same percentage of unfermented legume flours (Rizzello, Calasso, et al., 2014). Compared to native legume flours, texture instrumental analysis demonstrated that sourdough fermentation improved the bread softness (hardness decreased by ca. 30%) and crumb elasticity. Resilience, springiness and cohesiveness of breads fortified with fermented legume flours were comparable to those of conventional wheat flour bread. The addition of buckwheat sourdough strengthened the gluten network and decreased elasticity (Moroni, Zannini, Sensidoni, & Arendt, 2012). Wheat bread formulations with up to 10% incorporation of brewer's spent grains fermented with sourdough resulted in dough with improved handling properties (Waters et al., 2012). Bread fortified with sourdough

fermented bran had higher specific volume, lower resilience and cohesiveness, and higher hardness, gumminess and chewiness than wheat bread made with baker's yeast (Pontonio et al., 2020). Sourdough fermentation overcame the quality losses in sugar-reduced cakes, biscuits and burger buns allowing the similar specific volume of full-sugar control and contributed to softer crumb (Sahin et al., 2019). Other research articles (30) addressed the rheology of gluten-free breads made with buckwheat, chia, sorghum, teff, chestnut, quinoa and other gluten-free matrices. Rheology improvements were observable using sourdough fermentations, in particular with *L. plantarum* (Moore et al., 2008) and *L. amylovorus* (Axel et al., 2015).

7.2. Sensory attributes

Most of the research articles (180) faced descriptive panel analyses, while others (47) deepened the sensory attributes through the determination of volatile components (VOC). Comparing sourdough vs. baker's yeast breads and merging research articles that used the same descriptive approach up to 27 sensory attributes made possible the differentiation (Fig. 5). Acidic taste and smell, intense aftertaste and aroma, attractiveness, pronounced crumb and crust color, crust crispness, freshness, fruitiness, high porosity and sourness were the main sensory attributes, which clearly described the uniqueness of sourdough breads. If these are the main sensory traits, they combine with VOC of various chemical classes. Overall, mass spectrometry analyses identified ca. 90 VOC in sourdough breads (mainly from wheat flour): alcohols, carbonyls (aldehydes and ketones), esters, acids and miscellaneous components (Fig. 6). We elaborated the dataset from 47 research articles drawing a heat-map, which correlates the VOC prevalence to the dominant sourdough lactic acid bacteria and yeasts. The synthesis of VOC is clearly species specific, being evident how *Lactobacillus acidophilus*, *L. brevis*, *Lactobacillus curvatus* (*Latilactobacillus curvatus*), *L. fermentum*, *Lactobacillus helveticus*, *Lactobacillus sakei* (*Latilactobacillus sakei*), *L. sanfranciscensis* and, mainly, *L. plantarum* contribute to higher

and wider spectrum of VOC with respect to baker's yeast. The liberation of FAA (e.g., Phe, Leu, Cys and Orn) per se contributed to bread flavor (Thiele, Gänzle, & Vogel, 2002). The conversion of Glu to Gln by *L. sanfranciscensis* and *Lactobacillus reuteri* (*Limosilactobacillus reuteri*) increased their acid tolerance and, concomitantly, affected the dough flavor (Vermeulen, Gänzle, & Vogel, 2007). Cell-free extracts from sourdough lactic acid bacteria were essential sources of glutamate dehydrogenase and cystathionine- γ lyase, which synthesized key VOC during sourdough fermentation (Cavallo et al., 2017). The sourdough fermentation with *L. reuteri* converted FAA to γ -glutamyl dipeptides, which improved the taste intensity (Zhao & Gänzle, 2016). The unequivocal conclusion was that sourdough confers a unique and superior flavor and taste, especially because of the liberation of FAA during fermentation, which act as precursors of VOC or directly affect the flavor intensity.

7.3. Shelf life

Staling and fungal contamination are the main causes for decreasing the shelf life of baked goods, whose relevance varies depending on the product and duration of storage. Compared to fermentation by baker's yeast, sourdough *L. sanfranciscensis* and *L. plantarum* delayed bread staling by decreasing the rate of firmness and starch retro-gradation (Corsetti, Gobbetti, Balestrieri, et al., 1998). The use of a selected sourdough targeting pentosan hydrolysis delayed bread firmness and staling (Corsetti et al., 2000). The combination of wheat bran, enzymes (α -amylase, xylanase and lipase) and sourdough exhibited least changes in crumb firmness, amylopectin crystallinity and rigidity of polymers, which all delayed staling (Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006). The synergistic effect of sourdough and transglutaminase, an enzyme able of catalyze the formation of protein cross-links resulting in extensive nets, was also promising (Scarnato et al., 2017). In other cases, a delayed staling was observable combining the sourdough fermentation with non-wheat ingredients such as wheat

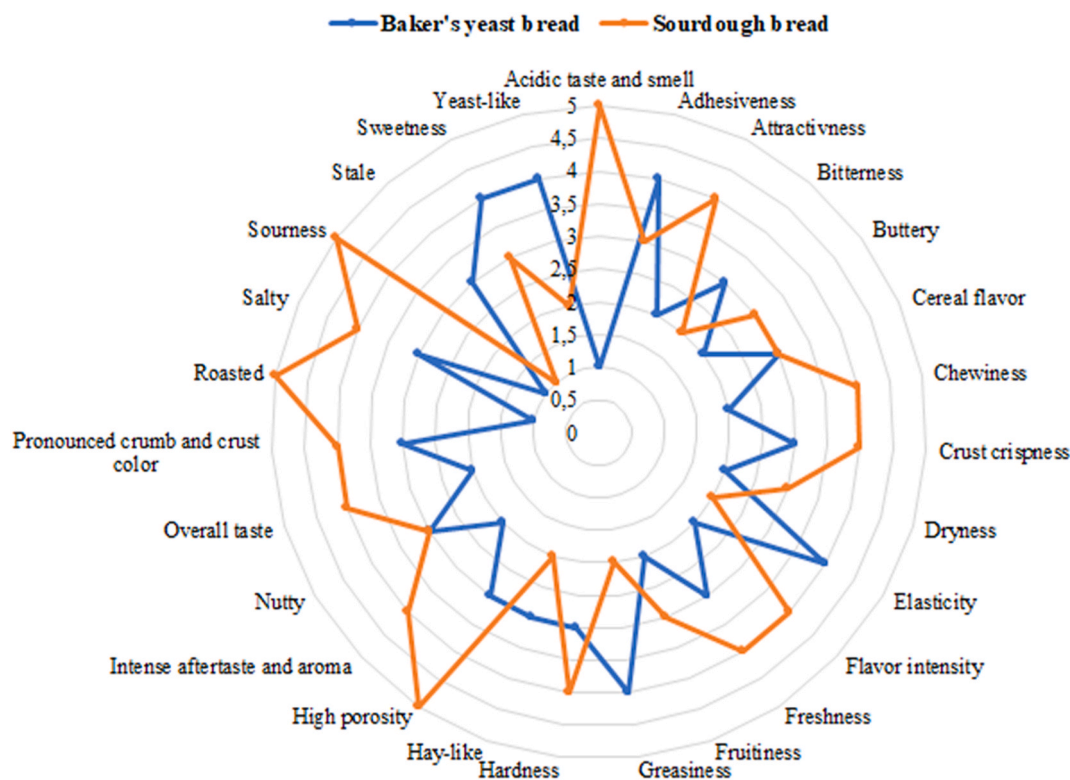


Fig. 5. Summarized characteristics and respective average scores based on descriptive sensory analyses (48 research articles) of sourdough vs. baker's yeast breads as assessed by trained panelists.

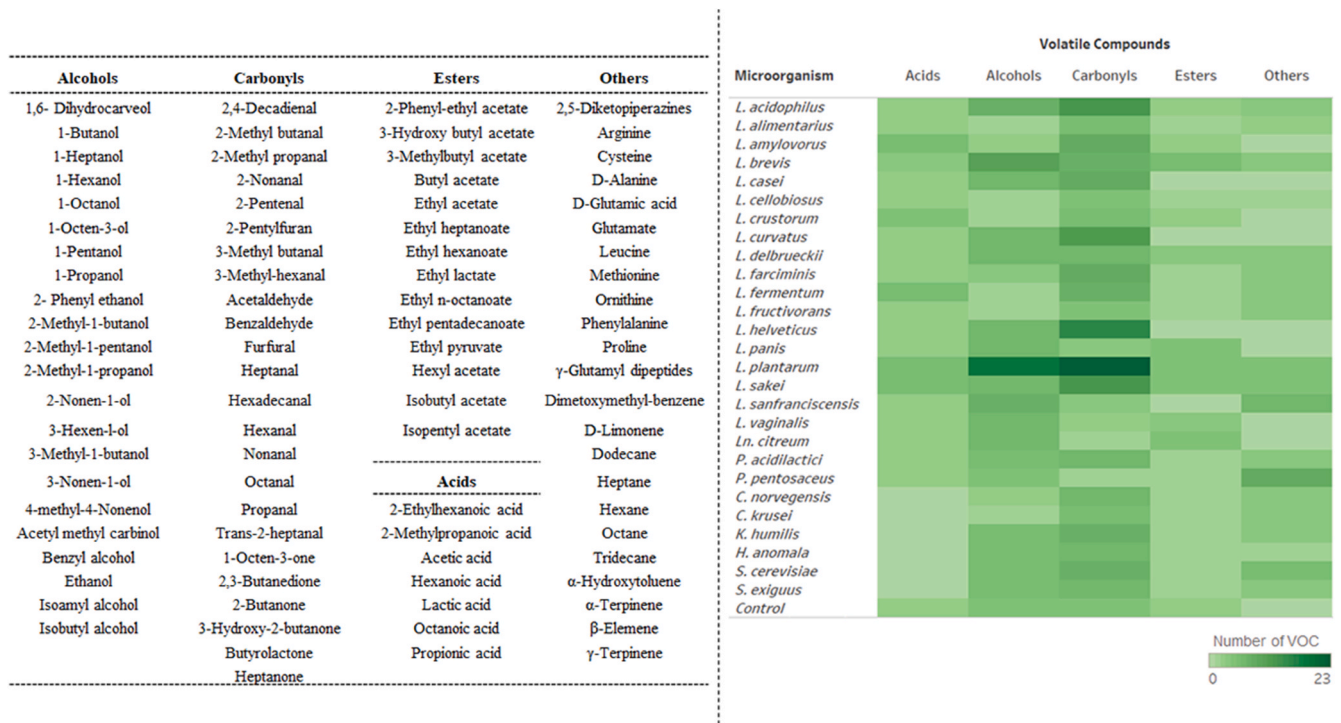


Fig. 6. List of volatile components (VOC) (left) identified in sourdough breads by mass spectrometry techniques and VOC profiles (right) as determined using single strains to start the sourdough fermentation. The comparison is with respect to baker's yeast bread (control).

germ (Rizzello, Cassone, Coda, & Gobbetti, 2011) or flaxseeds (Quattrini et al., 2019), and millet (Wang et al., 2019) and chestnut (Rinaldi, Paciulli, Caligiani, Scazzina, & Chiavaro, 2017) flours. Although neither the staling mechanisms nor the microbial activities were completely understood, the incontestable evidence is that sourdough baked goods have delayed staling.

With the extension of the shelf life for responding to consumer expectations, the fungal contamination became the major cause of spoilage for baked goods. Concomitantly, the reduction, or better, the elimination of chemical preservatives was another issue raised by industries. Twenty-five years ago, a pioneer research article already proved the capability of sourdough fermentation to some extent inhibit fungal spoilage through the synthesis of a mixture of acetic, caproic, formic, propionic, butyric and n-valeric acids (Corsetti, Gobbetti, Rossi, & Damiani, 1998). Later on, phenyllactic and 4-hydroxy-phenyllactic acids, which also acted as antifungal compounds, were identifiable during sourdough fermentation with *L. plantarum* (Lavermicocca et al., 2000). Other strains of *L. plantarum* also synthesized cyclic dipeptides (L-Leu-L-Pro and L-Phe-L-Pro) with antifungal activities (Dal Bello et al., 2007). A very abundant literature regarding the antifungal properties of other lactic acid bacteria species, likely *L. amylovorus* (Axel et al., 2015), *L. paracasei* (Mantzourani et al., 2019), *Lactobacillus hammesii* (*Levilactobacillus hammesii*) (Quattrini et al., 2019), and *L. reuteri* (Axel et al., 2016), succeeded. Supplementary Fig. 6 lists the antifungal compounds variously discovered during time. The list includes 34 carboxylic acids, and 31 proteins and peptide derivatives liberated during sourdough fermentation or derived from vegetable and water-soluble extracts of flours, and used in combination with sourdough. The current trend is to combine the inhibitory activities from lactic acid bacteria, yeasts (e.g., ethyl acetate from *Wickerhamomyces anomalus* and *Meyerozyma guilliermondii* (Coda, Cassone, et al., 2011; Coda et al., 2013) and natural matrices (e.g., legumes, flours, milling by-products and essential oils) (Debonne, Van Bockstaele, De Leyn, Devlieghere, & Eeckhout, 2018; Ricci et al., 2019; Rizzello, Lavecchia, Gramaglia, & Gobbetti, 2015), and using innovative active packaging technologies with oxygen absorbers or antimicrobial releasers (Noshirvani, Ghanbarzadeh, Rezaei

Mokarram, & Hashemi, 2017). A number of research articles (e.g., Rizzello, Lavecchia, et al., 2015; Ryan et al., 2011) showed how this bio-preservation, at semi-industrial or industrial plants, allowed an extension of the shelf life for weeks with an antifungal activity similar or better than that of chemical preservatives. Further efforts in this direction are warranted to manufacture long shelf life leavened baked goods free from chemicals, which reflects the main consumer expectations.

8. Nutritional attributes

Once demonstrated conclusive effects on sensory, rheology and shelf life attributes, most of the research activities moved forward nutritional aspects. We retrieved 527 research articles, with a relevant temporal increase from 2005 to 2009 to 2015–2019, having in this last interval the highest peak of 231 publications. Nutritional attributes mainly concerned sourdough breads made with various types of flours. The world cloud of Fig. 7 shows the nutritional features faced during time. We decided to review systematically those issues that are more consistent.

8.1. Mineral bioavailability

Phytic acid (myo-inositol hexaphosphate) is a natural constituent of cereals, pseudo-cereals and legumes, where it forms insoluble complexes with minerals and other compounds, thus decreasing their dietary bioavailability/bioaccessibility (Martínez et al., 1996). Enzymes responsible for the hydrolysis of phytic acid are phytases (myo-inositol hexakisphosphate phosphohydrolase; EC 3.1.3.8/EC 3.1.3.26), which sequentially release soluble inorganic phosphate, low size inositol phosphate and myo-inositol. Research articles (103), mainly from the last decade, approached this issue determining the residual content of phytic acid or the mineral, mainly iron, bioavailability in doughs and breads subjected to sourdough fermentation. A marked increase of the mineral bioavailability resulted because of the sourdough acidification, which indirectly activates the flour endogenous phytases, and the microbial enzyme activities. In general, the most suitable level of



Fig. 7. Word cloud representing the nutritional attributes focused in the last 30 years as influenced by sourdough fermentation.

acidification is in the range 4.3–4.6 and decreases in phytic acid content are above 70% (Larsson & Sandberg, 1991). A large spectrum of minerals became bioavailable, mainly including calcium, sodium, magnesium, iron, and zinc (Di Cagno et al., 2008). The literature describes 30 species of lactic acid bacteria and 5 species of yeasts, and an overall number of 146 strains, which, presumptively, harbor phytase activities (Supplementary Table 2). Eighteen species are only belonging to the *Lactobacillus* genus. Although most of these research articles did not demonstrate the presence of the enzyme and an indirect activation of the flour endogenous phytases might had overlapped the microbial activities, all data emphasized how the sourdough fermentation is the unique tool for increasing the mineral bioavailability of baked goods made with cereal, pseudo-cereals and legumes.

8.2. Dietary fibers

The World Health Organization recommends a DF daily intake of 25 g/day, but the effective consumption is markedly lower. Dietary interventions for increasing the DF intake are, therefore, desirable. We retrieved 60 research articles dealing with the effect of sourdough fermentation on total DF, ratio between water-soluble and -insoluble DF, and individual fractions. Although cereals and pseudo-cereals per se are sources of DF (e.g., hemicellulose, resistant starch, β -glucans, arabinoxylans) (Williams, Mikkelsen, Flanagan, & Gidley, 2019), the common strategy was to increase the DF content of baked goods, including gluten-free products, through the fortification with various percentages of bran (5–20%), wheat germ (4–7.5%), brewer's spent grains (5–20%) or mixing cereal, pseudo-cereal and legume flours. Nevertheless, the modifications of the traditional recipes negatively affect the sensory and rheology attributes, and the ratio between water-soluble and -insoluble DF, in several cases, needs some changes. The use of sourdough faced all these aspects. Emblematic research articles demonstrated that sourdough fermentation allowed the fortification with bran up to the concentration of 20% (Salmenkallio-Marttila, Katina, & Autio, 2001), the increase of DF in almost all gluten-free products (Di Cagno et al., 2008) and incremented the aliquot of water-soluble DF in cereal and legume mixtures (Chinma et al., 2016). Furthermore, it allowed the exploitation of matrices naturally rich in DF (e.g., fava bean, hemp) (Wang et al., 2018) without compromising the sensory and rheology features of baked goods.

8.3. Glycemic index

Glycemic index (GI) is a numerical value assigned to foods based on their capability to increase the blood glucose levels after consumption. According to the Harvard Medical School, foods rank into high (≥ 70), moderate (between 69 and 55) and low (≤ 55) GI. The calculation of GI in foods introduces the concept of Glycemic Load (GL), which estimates how the quantity of carbohydrates in foods raises the blood glucose levels depending upon the type of carbohydrate present in that food and, thus, each food (or carbohydrate) exhibits different glyceamic response (Eleazu, 2016). The literature shows 52 research articles focusing on GI or GL as influenced by sourdough fermentation; 40 dealt with *in vivo* challenges and 12 used *in vitro* approaches. Overall, *in vivo* challenges recruited healthy volunteers, with numbers ranging from 15 to 25 and an average age of 20–60 years. Volunteers, mostly under double blind conditions, consumed sourdough bread or control bread started with baker's yeast, after an overnight fasting of 10–12 h. Before analyses, the collection of blood samples was every 15 min; within an overall timing of 2–3 h. Using data from 22 *in vivo* challenges, Fig. 8 shows the box plots for GI of sourdough vs. baker's yeast breads. Median values clearly indicate how only the sourdough fermentation has the capability to shift the bread GI from high to moderate. The same trend was observed for gluten-free products where excess of calories and carbohydrates are the main nutritional constraints (Wolter, Hager, Zannini, & Arendt, 2014). When sourdough fermentation combines with the addition of DF (5%–10%), the GI decreases to values lower than 55, which rank these baked goods as low GI foods, recommendable for all dietary habits. Apart from *in vivo* or *in vitro* approaches, the main issue, from the pioneer study of Liljeberg, Lönner, and Björck (1995) to the last reports (e.g., Rizzello et al., 2019), was not only to demonstrate the lowering of GI but also to explain the mechanisms behind. Biological acidification (Liljeberg & Björck, 1998), increased resistant starch (Liljeberg, Åkerberg, & Björck, 1996), liberation of peptides, FAA, polyphenols and water-soluble DF (Nilsson, Östman, Preston, & Björck, 2008), fast gastric emptying, stimulation of satiety hormones (Rizzello et al., 2019) and use of fermentable cereal, DF and legumes mixtures were all factors/interventions that, also concomitantly, improved this nutritional attribute. Recent advances in clinical studies show that GI and GL responses after bread ingestion also rely on gut microbiome functionality, which highlights the importance of personalized dietary recommendations (Korem et al., 2017), and suggests to assess the effects sourdough baked goods on gut microbiome composition and functionality (under investigation in the author's laboratory).

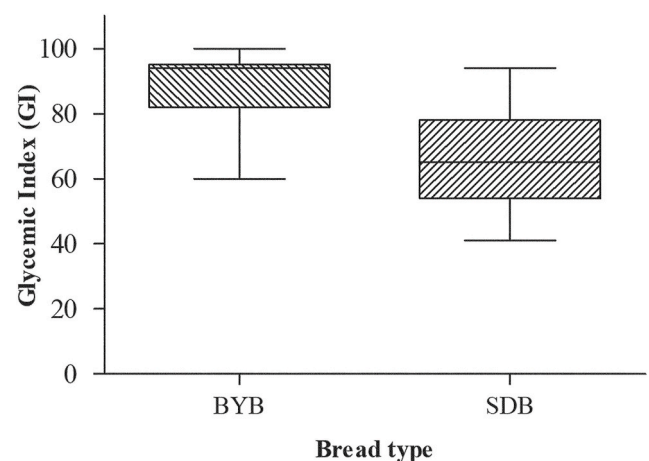


Fig. 8. Box plot showing the values of glycemic index (GI) of sourdough breads (SDB) vs. baker's yeast breads (BYB). Median values are 65.1 and 94.2, respectively. The scale for GI is from 0 to 100. The calculation was from 22 research articles dealing with *in vivo* challenges.

8.4. Protein digestibility

Empirical and *in vitro* scientific evidences all agree that sourdough fermentation associates to an improved bread digestibility, mainly related to proteins. The literature shows 27 research articles dealing with this issue, 25 using *in vitro* approaches and only 2 setting *in vivo* challenges. Almost all *in vitro* investigations concluded that the *in vitro* digestibility of protein (IVPD), expressing the stability of protein hydrolysates and how they withstand digestive processes, increased with sourdough fermentation. This apart from the flours and products. Other indices improved with sourdough, especially under prolonged fermentation. In particular, the amount of protein required to provide the minimal essential amino acid pattern (chemical score, CS); the protein nutritional quality based on the amino acid profile after hydrolysis (protein efficiency ratio, PER); and the nutritional index (NI), which normalizes the qualitative and quantitative variations of the protein compared to its nutritional status. Recently, also the addition of dried fruits was a suitable technological option for increasing the content of essential amino acids. For instance, the pistachio powder added to flour or semolina remarkably increased the content of lysine in sourdough baked goods (Gaglio et al., 2020). The sourdough fermentation with the addition of dried pear and orange resulted in a significant increase of the FAA concentration, including two essential amino acids such as valine and methionine (Yu, Wang, Qian, Zhang, & Qi, 2018). Skrede, Sahlström, Ahlström, Connor, and Skrede (2007) used mink (*Mustela vison*) as an animal model to demonstrate that sourdough fermentation had positive nutritional implications by limiting the effects of anti-nutrients, and improving digestibility and energy utilization. Rizzello et al. (2019) recruited 36 healthy volunteers who underwent an *in vivo* challenge in response to bread ingestion. Sourdough bread with moderate acidification stimulated more appetite and induced lower satiety. The sourdough bread with most intense acidic taste induced the highest fullness perception in the shortest time. Gall bladder response did not differ among breads, while gastric emptying was faster with sourdough vs. baker's yeast breads. Oro-cecal transit was prolonged for baker's yeast bread and faster for sourdough breads, especially when made with long-time fermentation whose transit lasted ca. 20 min less than baker's yeast bread. Differences in carbohydrate digestibility and absorption determined different post-prandial glycaemia responses. Sourdough breads showed the lowest values. After ingesting sourdough breads, which had the highest total FAA content, the levels of FAA in blood plasma maintained constantly at high levels for extended time. While the improvement of the digestibility is evident, the mechanisms promoting this are less definable. Biological acidification per se or through the indirect activation of flour endogenous proteases, secondary proteolysis through a portfolio of lactic acid bacteria peptidases and, more in general, modification of the gluten network, which becomes more susceptible to digestive enzymes, are some plausible explanations.

As the triggering factor for several disorders, gluten as well was targeted. A number of research articles (41) aimed at exploiting the potential of sourdough fermentation for its degradation. The main evidences concerned the elimination of traces of gluten, which prevented cross contamination in gluten-free products (Di Cagno et al., 2008), the partial hydrolysis of gluten ((Rizzello et al., 2014)), which improved the digestibility, and the full gluten digestion for rendering gluten-free baked goods made with soft or durum wheat flour (Rizzello et al., 2007). Selected strains of lactic acid bacteria, combined with food-grade fungal proteases, were capable of decreasing the residual content of gluten to less than 10 ppm under semi-liquid sourdough fermentation lasting ca. 24 h. Three clinical challenges (Di Cagno et al., 2010; Greco et al., 2011; Mandile et al., 2017), based on immunological and serological analyses and biopsy specimens on celiac patients, demonstrated the absolute safety of baked goods made with fully hydrolyzed soft and durum flours.

8.5. Degradation of anti-nutritional factors

Although very rich in nutrients, cereals, pseudo-cereals and legumes also contain anti-nutritional factors (ANF), which in part limits their consumption or cause severe disorders. Apart from phytic acid (already discussed in paragraph 8.1), raffinose, condensed tannins, vicine and convicine, saponins and trypsin inhibitors are the main ANF, whose presence and amount depend on the vegetable matrix. Raffinose is not digestible by pancreatic enzymes but fermentable by gas-producing bacteria in the large intestine, causing gut disorders. Condensed tannins and trypsin inhibitors inhibit digestive enzymes leading to poor digestibility of proteins and other nutrients. Biologically active glycosides such as saponins, vicine and convicine cause the hemolysis of red blood cells and form complexes with nutrients, preventing their absorption. In particular, vicine and convicine are precursors of the aglycones divicine and isouramil, the main causing agent of favism, a genetic condition leading to severe hemolysis after fava bean ingestion. While heat treatments fully inactivate trypsin inhibitors, the others ANF are heat resistant. De-hulling, soaking, germination, air classification and extrusion are only in part effective in decreasing the content of ANF, therefore, other options, including sourdough fermentation, underwent investigation. We retrieved 58 research articles dealing with the capability of sourdough fermentation to degrade ANF. Apart from lactic acidification, mainly sourdough lactic acid bacteria harbor a portfolio of enzymes, likely α -galactosidase, β -glucosidase and tannases, which have the potential to counteract the presence of several ANF. Sourdough fermentation with selected *L. plantarum* fully degraded vicine and convicine within 48 h, with aglycone derivatives not detectable (Rizzello, Losito, et al., 2016). *Ex-vivo* assays on human blood confirmed the lack of toxicity of sourdough fermented fava bean. The sourdough fermentation of whole grains of wheat, barley, chickpea, lentils and quinoa, and yellow and red lentil, white and black bean, chickpea, and pea flours decreased the concentrations of raffinose (62–80%), condensed tannins (23%), trypsin inhibitors (23–44%) and saponins (68%) (Montemurro, Pontonio, Gobetti, & Rizzello, 2019). The combination of gelatinization and sourdough fermentation further lowered the residual concentrations of condensed tannins (62%) and trypsin inhibitors (70%) (De Pasquale, Pontonio, Gobetti, & Rizzello, 2020). Literature data came to the convergent belief that, as mild and cost-effective bioprocessing, the sourdough fermentation is the most promising option to degrade a large spectrum of ANF for industrial applications.

Although the fundamental research has yet to discover some of the sourdough potential dealing with nutritional attributes, currently it would be worthwhile to correctly and convincingly deliver the above nutritional findings both to industries and, especially, consumers.

9. Conclusions

Almost 30 years of research activity on sourdough fermentation, with more than 1200 research articles published, is a suitable time to get some conclusions, which would represent milestones for scientists, industries and consumers. Because of its unique and complex microbial composition, which establishes itself with the baker care, the sourdough has undoubted advantages with respect to any other leavening agents, in terms of sensory, rheology, shelf life and multiple nutritional attributes. While further nutritional features need consolidation or discovering, one of the interim prospects would concern the investigation of the complex metabolic interactions among dominant lactic acid bacteria and yeasts and less abundant satellite members, which should depict what we may define the sourdough fermentome. This will improve the performance and, at the same time, will favor longer stability and shorter time of fermentation, which certainly will spread the use at artisanal and industrial levels.

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Author contributions

Kashika Arora: Conceptualization, Investigation, Writing - original draft preparation. Hana Ameer: Conceptualization, Investigation, Writing - original draft preparation. Andrea Polo: Conceptualization, Investigation, Writing - review and editing. Raffaella Di Cagno: Writing - review and editing. Carlo Giuseppe Rizzello: Writing - review and editing. Marco Gobbetti: Conceptualization, Investigation, Writing - original draft preparation, Supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tifs.2020.12.008>.

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