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Functional and biochemical characterization of pre-fermented ingredients obtained by the fermentation of durum wheat by-products

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ABSTRACT

This work was aimed to characterize functional and biochemical parameters of a bakery ingredient prepared with durum wheat by-products (micronized bran and middling) fermented by a selected microbial consortium composed of yeasts and lactic acid bacteria. The unfermented milling by-products mixture and the mixture fermented by a baker's yeast were used as reference. The innovative ingredient showed more stable colour indexes compared to the references, a more complex profile in volatile molecules characterized by a higher presence of alcohols, ketones and acids compared to the references. A significant increase in the content of peptides, short chain fatty acids, total phenols, antioxidant activity and prebiotic activity together with a reduction in phytic acid content was observed in the samples fermented by the selected microbial consortium compared to the references. This work provides information on the impact of lactic acid bacteria and yeasts on functional and biochemical characteristics of fermented milling by-products.

1. Introduction

Wheat is the most extensively grown cereal crop worldwide, both in terms of kernel yield and cultivated area. Currently, the most significant varieties of wheat are hexaploid soft wheat (*Triticum aestivum* L.), primarily used in the production of wheat-based foods such as bread and biscuits, and tetraploid durum wheat (*Triticum durum* Desf.), especially used for pasta production (de Sousa et al., 2021). According to FAOSTAT (2022), global wheat production reached 808 million tons (Mt) in 2022, with durum wheat accounting for approximately 30 Mt. Traditionally milling processes are based on the isolation and utilization of endosperm, which is primarily made of starch and proteins. Therefore, the outer husks and germ are typically discarded, representing approximately 23 %–27 % of the milling output (Wrigley et al., 2015; Nayik et al., 2023; Cardenia et al., 2018; Prückler et al., 2014). However, wheat-milling by-products represent a valuable source of dietary fibers, antioxidants, B vitamins and minerals (Stevenson et al., 2012). Certain health benefits associated with wheat bran have been already acknowledged by the European Food Safety Authority, including its capacity to promote intestinal transit and increase faecal mass (Shewry, 2009; EFSA Panel on Dietetic Products and Nutrition and Allergies (NDA), 2010). Moreover, epidemiological evidence suggests that daily consumption of cereal products enriched with bran may reduce the risk of colorectal cancer, cardiovascular disease, coronary atherosclerosis, obesity and type 2 diabetes (Cheng et al., 2022; Singh et al., 2019; Vuksan et al., 2020; de Munter et al., 2007). Despite the growing industrial and consumer interest in the inclusion of wheat bran into cereal food products, its current utilization remains limited in comparison to its production rate and the technological and functional potential of these by-products is currently not being fully exploited (Luithui et al., 2019). Indeed, despite their excellent functional properties, incorporating wheat bran into cereal-based foods still presents challenges due to its negative technological and sensory impact (Siroli et al., 2022; Alzuwaid et al., 2021). For example, inclusion of wheat bran in bakery products can have adverse effects on dough rheology, resulting in reduced loaf volume, impaired bread crumb texture, and imparting a dark colour and undesirable flavour to foods (Alzuwaid et al., 2021; Cai et al., 2014).

To enhance the nutritional, technological and functional properties

Abbreviations: DFM, Durum fermented mixture; UFM, Unfermented milling by-product mixture; BCM, Benchmark control mixture; LAB, Lactic acid bacteria.

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of bran, new milling techniques, enzymatic treatments, and fermentation processes have been developed and studied (Coda et al., 2015). For example, Pasqualone et al. (2017) showed that the negative effect of the bran addition in bread making was mitigated by its micronization. In fact, the bread obtained by the addition of micronized durum wheat bran showed similar properties compared to the pure re-milled semolina bread (Pasqualone et al., 2017). Moreover, in recent years, the fermentation of wheat bran attracted interest from the scientific community. Indeed, the metabolic activity of selected microorganisms or microbial consortia can enhance its technological and health-promoting properties (Bertsch et al., 2020; Verni et al., 2019). For instance, several studies have demonstrated an increase in phytase activity during bran fermentation, resulting in reduced levels of phytic acid (Zhang et al., 2022; Coda et al., 2014; Manini et al., 2014; Zhao et al., 2017). Fermenting wheat bran with selected microbial consortia, containing yeast and lactic acid bacteria, can also increase the concentration of peptides and free amino acids, including the functional γ-aminobutyric amino acid (GABA), thereby enhancing the *in-vitro* digestibility of proteins (Arte et al., 2015; Coda et al., 2014; Manini et al., 2014). Furthermore, incorporation of fermented wheat bran, whether fermented with yeast or lactic acid bacteria, in bakery products has been demonstrated to increse the phenolic content and consequently enhance the antioxidant activity of bread (Katina et al., 2012; Pontonio et al., 2017; Prückler et al., 2015). A microbial consortium containing *Latilactobacillus curvatus*, *Kazachstania servazzii* and *Kazachstania unispora,* utilized for fermenting a mixture of wheat germ and rye bran, demonstrated the ability to enhance the overall properties of the fermented product (Siroli et al., 2022). Therefore, employing well-characterized microbial consortia for fermenting high fiber ingredients, such as wheat bran mixtures or sourdough, could enhance their functional, qualitative, and technological characteristics.

Despite most of the available literature focuses on enhancing by-products of soft wheat, rice, barley and rye, few studies address durum wheat bran, which nevertheless represents a by-product of great impact in the Mediterranean area (Spaggiari et al., 2021; Galanakis, 2022; Bartkiene et al., 2020; Sabater et al., 2020). Regarding durum wheat, recent studies have indicated that edible oil obtained from durum wheat by-products may serve as a rich source of phytochemicals. This suggests a promising approach for maximizing the utilization of durum wheat by-products (Squeo et al., 2022). However, as demonstrated with other agrifood by-products, a targeted fermentation of durum wheat by-products may modify their overall characteristics.

In this context, this research aimed to demonstrate how a low-value by-product, such as durum wheat bran, can be enhanced through targeted fermentation processes, thereby increasing its potential in food applications. In this study, a selected microbial consortium composed of lactic acid bacteria and yeasts was used to ferment a mixture of durum wheat by-products fractions. The effect of fermentation, by the selected microbial consortium, on functional and biochemical characteristics of the milling by-products mixture was compared with a mixture fermented by a commercial baker's yeast and an unfermented milling by-product mixture.

2. Material and methods

2.1. Raw material: durum wheat by-products fractions

Barilla G. e R. F.lli S.p.A. (Parma, Italy) supplied fractions of durum wheat by-products, comprising durum wheat micronized bran and durum wheat middling. The by-products fractions were characterized for protein, humidity, ash, total, soluble and insoluble dietary fibers, total fat and mineral content according to Khalid et al. (2017). The starch content and fatty acid profiles were determined according to AACC (2000) and AOAC International respectively. Lipid fractions were evaluated by Gas Chromatography - Mass Spectrometry analysis according to AACC (2000). Phytate content was investigated according to Buddrick et al. (2014). Each of the reported analyses was performed in triplicate.

2.2. Microbial consortium

The microbial consortium, used as starter for the fermentation of milling by-products, was composed by three lactic acid bacteria (LAB), i.e. *Latilactobacillus curvatus* LANC A, *Leuconostoc mesenteroides* LANC B and *Pediococcus pentosaceus* LANC C, and two yeast strains, i.e. *Kazachstania servazzii* KAZ2 and *Kazachstania unispora* FM2. All strains of the selected microbial consortium belong to the collection of the Department of Agriculture and Food Sciences of the University of Bologna and were isolated from spontaneously fermented milling by-products. The selection of this specific microbial consortium was based on preliminary tests, carried out in the framework of the EU project INGREEN (Siroli et al., 2022). First, following spontaneous fermentation of different milling by-products was possible to identify several microbial consortia, composed of yeast and lactic acid bacteria, responsible for the fermentation process. The most performing microbial consortium, used in this work, was selected based on the fastest fermentation kinetics on durum wheat milling by-products fractions and the capability to provide an increase of functional and organoleptic characteristics such as antioxidant activity and volatile molecule profile.

The yeast and LAB strains, stored as frozen stocks (−80 °C) were pre-cultivated three times, prior to be used, on Yeast Extract–Peptone–Dextrose (YPD) medium (Oxoid, Italy) for *Kazachstania* spp. and on Maltose, de Man, Rogosa and Sharpe (mMRS) medium (Oxoid, Italy) for LAB and incubated at 25 °C for 48 h and 24 h respectively for yeasts and LAB.

2.3. Fermentation of durum wheat by-products

The milling by-product mixture used in this work was composed by durum wheat middlings (75 %) and durum wheat micronized bran (25 %) and was prepared according to Siroli et al. (2022). One kilogram of the mixture was produced in a commercial kneader (Major, Kenwood, Italy) by combining durum wheat middlings (75 %) and durum wheat micronized bran (25 %), hydrated with tap water in a 2:1 ratio. The composition of the mixture was determined based on the compositional characteristics of the by-products and the ability of the microbial consortium to exhibit rapid fermentation kinetics and to maintain stability over time.

The LABs and yeasts strains were pre-cultivated as reported in section 2.2 and then cultured on mMRS broth for 24 h at 25 °C and on YPD broth for 48 h at 25 °C, for LABs and yeasts respectively. Prior to use, the strains were centrifuged at 10,000 rpm and washed twice with saline solution (0.9 % NaCl).

The hydrated milling by-product mixture was inoculated with 7.0 log CFU/g of LABs and 4.5 log CFU/g of yeasts. The inoculated mixture was statically fermented at 25 °C for 24 h in a sanitized tank. The innovative ingredient fermented by the microbial consortium of yeasts and LAB, referred to as "durum fermented mixture" (DFM), was compared with two control samples: an unfermented milling by-product mixture (UFM) and a benchmark control mixture fermented by commercial baker's yeast (BCM), produced using the same milling by-product mixture but inoculated with lyophilized commercial baker's yeast (Lievital, Lesaffre Italia, Parma, Italy) at an initial concentration of 7.0 log CFU/g. Before use, the baker's yeast was rehydrated by adding water at 30 °C for 15 min. Fermentation experiments were carried out in triplicate on different days.

2.4. Acidification kinetics and plate count

The acidification kinetics were monitored by measuring the pH of the different preferments every two hours from 14 to 24 h of fermentation at 25 °C. The cell counts of LAB and yeasts were determined at the

beginning (0 h) and at the end (24 h) of fermentation by plate counting on selective agar media.

Yeast enumeration was conducted using YPD agar supplemented with 0.02 % chloramphenicol, while LAB cell counts were determined on m-MRS agar supplemented with 0.02 % cycloheximide. Additionally, the total titratable acidity was determined at the end of fermentation following the method described by Rizzello et al. (2010).

2.5. Nutritional profile

According to the Reg UE 1169/2011 25/10/2011 GU CE L304 22/11/2011, the preferments were characterized for their energy, fats, saturated fatty acids, carbohydrate, sugar, dietary fiber, protein, and salt content.

Specifically, the energy content was evaluated according to da Rocha Lemos et al. (2021). The fat content was determined using the Soxhlet extraction method described in AOAC official methods (Aoac 920.39, 2022), and saturated fatty acids were analysed as previously described by dos Santos Oliveira et al. (2011). The carbohydrate content (%) was calculated by subtracting the ash, fat, fibre and protein content from total dry matter (Costantini et al., 2014). Sugar content was measured according to the method outlined by Luchese et al. (2015), while dietary fibers was evaluated using the AACCI Approved Method 32–07.01, as reported by Khalid et al. (2017). The protein content was determined using the Kjeldahl method, with a conversion factor of N × 5.7. The acetic acid and lactic acid content were measured using the acetic acid assay kit and lactic acid assay kit, respectively (Megazyme, Ayr, UK). The fermentation quotient was calculated as the molar ratio between lactic and acetic acids.

2.6. Colour analysis

The colour was assessed according to Siroli et al. (2022), using a Minolta® CR-400 colorimeter (Milan, Italy) that was previously calibrated. The CIELAB system was utilized, with parameters including lightness (L*), redness (a*) and yellowness (b*), which were used to objectively define the colour (Pointer, 1981). Colour determination was conducted for each preferment both before and after 24 h of fermentation.

2.7. Volatile molecule profiles and short-chain fatty acids

The volatile compounds of both the pre-fermented and unfermented mixtures were analysed using solid phase micro extraction-gas chromatography-mass spectrometry (SPME-GC/MS) following the method outlined by Burns et al. (2008), with some modifications as reported by Rossi et al. (2021). The analysis was conducted with an Agilent Technology 7890N gas chromatograph coupled with a Network Mass Selective detector HP 5975C mass spectrometer (Agilent Technologies).

2.8. Fatty acid profiles

The lipid fraction extraction was performed according to the method reported by Boselli et al. (2001), with some modifications as described in Rossi et al. (2021). The composition of fatty acids was determined as fatty acid methyl esters (FAMEs) using GC-MS. Methyl tridecanoate (Sigma, Milan, Italy) was used as internal standard (13:0, 0.02 mg/mL), and Supelco FAME MIX 37 (Sigma) served as the external reference. The total profiles of fatty acid methyl esters were analysed following the procedure described by Rossi et al. (2021).

2.9. Peptide, phytate content and protein profile

The peptide content was determined in sample extracts obtained following the method outlined by Siroli et al. (2022), using the o-phtaldialdehyde (OPA) method as reported by Coda et al. (2012). A standard curve prepared with tryptone (0.1 to 1.5 mg/mL) was used as reference.

Phytic acid content was measured according to the AOAC 986.11/88 methodology (AOAC 986.11-, 2022), with a confidence interval calculated at a probability level of approximatively 95 %.

The protein profile was obtained using SDS–PAGE electrophoresis. Proteins were extracted from the different types of sourdough under reducing conditions following the method proposed by Marco et al. (2007), with some modifications as reported by Rossi et al. (2021). A quantity of 10 mg of the obtained pellet was mixed with 500 μL of Laemmli Sample Buffer 2X (Bio-Rad Laboratories, Milan, Italy) containing β-mercaptoethanol. The mixtures were incubated at 100 °C for 5 min, and then, 10 μL were diluted in 40 μL of Laemmli Sample Buffer. 20 μL of diluted samples were loaded on an 8–16 % Criterion TGX precast Gel (Bio-Rad Laboratories, Milan, Italy), with 10 μL of Precision Plus Protein Standard All Blue (Bio Rad) serving as standard. The gel was run in a Mini Protean Cell System with a Tris-Glycine SDS Running Buffer at 100 V for the first 10 min and then at 200 V for 1 h. Gels were stained and de-stained according to Gottardi et al. (2023). Images were captured using Bio-Rad's GS-900 (Bio-Rad, Milan, Italy).

2.10. Antioxidant activity and phenol content

The total polyphenols and antioxidant activity were assessed according to Siroli et al. (2022).

Antioxidant activity was evaluated using two different methods: the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay, as described by Rizzello et al. (2012), and the 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, according to Miller and Rice-Evans (1997). These determinations were conducted in triplicate. The concentration of total polyphenols was determined using the Folin–Ciocalteu method, as reported by Slinkard and Singleton (1977).

2.11. Prebiotic activity

To simulate passage through the gastro-intestinal tract, 100 mg of each sample (DFM, UFM and BCM) were digested in 3 mL of simulated gastric fluid (SGF, 125 mmol/L NaCl, 7 mmol/L KCl, 45 mmol/L $NaHCO_3$, 3 g/L pepsin, pH 2) for 3 h at 37 °C under stirring conditions (100 rpm). Subsequently, 3 mL of simulated intestinal fluid (SIF, 0.1 % w/v pancreatin, 0.15 % w/v Oxgall bile salt, pH 7) were added, pH was adjusted at pH 7.0 with NaOH 1 M, and digestion was carried out for further 3 h at 37 °C (100 rpm). Supernatants containing the digested and soluble parts of the pre-ferments were harvested after centrifugation (14.000 *g* for 20 min) and used to assess the prebiotic and antimicrobial activities.

The prebiotic activity of the samples was evaluated towards six *Bifidobacterium* spp. (*Bifidobacterium adolescentis* DSM20086, *Bifidobacterium bifidum* DSM20082, *Bifidobacterium breve* DSM20091, *Bifidobacterium longum* subsp. *infantis* DSM20090, *Bifidobacterium longum* subsp. *longum* DSM20219, and *Bifidobacterium angulatum* DSM20098) purchased from DSMZ (Braunschweig, Germany). Three mechanisms of action were considered: (i) stimulation of planktonic growth; (ii) stimulation of biofilm formation; (iii) stimulation of pre-formed biofilm.

Bifidobacteria were grown in de Man, Rogosa, and Sharpe broth (MRS, Difco, Detroit,MI, USA) supplemented with L-cysteine 0.05 % (w/v) (Merck, Milan, Italy) at 37 °C in anaerobic jars containing Gas-Pak EZ (Beckton, Dickinson and Co., Milan, Italy).

One hundred microliters of *Bifidobacterium* spp. suspensions in MRS (2×10^6 CFU/mL) were incubated in 96 multi-well plates (Corning Inc., Pisa, Italy) together with 100 μL of digested samples at 37 °C under anaerobic conditions. Wells inoculated with 100 μL of *Bifidobacterium* spp. suspension and 100 μL of the mixture of simulated fluids (SGF and SIF) were used as growth control. Blanks, consisting of simulated fluids and MRS, were also included. The plates were incubated for 24 h or 48 h to evaluate the impact on the planktonic growth and biofilm formation,

Table 1
Durum wheat by-products fractions used in this work.

Component %	Durum wheat bran micronized			Durum wheat middling		
Proteins %	16.30	±	2.00	20.00	±	0.50
Humidity %	11.40	±	0.30	12.10	±	3.00
Ashes %	4.70	±	0.20	4.60	±	1.10
Soluble Fiber %	3.30	±	0.90	2.90	±	1.20
Insoluble Fiber %	40.10	±	3.20	26.10	±	2.30
Total fat %	5.40	±	0.30	6.50	±	0.70
Starch %	15.00	±	3.00	23.00	±	2.50
Phytic acid %	2.50	±	0.10	2.60	±	1.00

respectively. *Bifidobacterium* spp. growth was quantified by reading the absorbance at 600 nm (EnSpire Multimode Plate Reader, PerkinElmer Inc., Waltham, MA, USA) and results are expressed in percentage with respect to growth control (100 %).

Biofilms were quantified through crystal violet (CV) staining as reported by Giordani et al. (2023) . Briefly, wells were washed twice, remaining biofilms were fixed in absolute ethanol (Merck) and stained with crystal violet 0.4 % (w/v) (Merck) for 10 min. The excess of dye was removed through three washing steps, CV bound to adherent cells was resolubilized in ethanol and the absorbance was measured at 595 nm. The biofilm formation was calculated as percentages relative to the absorbances of the control (100 %).

To investigate the effect on pre-formed biofilms, *Bifidobacterium* spp. biofilms were first allowed to form by seeding 200 μL of suspensions in 96 multi-well plates for 48 h. Afterwards, supernatants were removed, adherent cells were washed twice with sterile saline, and plates were further incubated for 24 h in the presence or not of samples as described above. Biofilms were finally stained with CV.

2.12. Antimicrobial activity against gastrointestinal pathogens

The antimicrobial activity of samples (DFM, UFM and BCM) was assessed against *Escherichia coli* SO107, *Escherichia coli* (ECET), *Salmonella enterica* and *Yersinia enterocolitica,* strains obtained from the Department of Pharmacy and Biotechnology of University of Bologna (Giordani et al., 2023).

All microorganisms were cultured in brain heart infusion broth (BHI) (Difco) at 37 °C under aerobic conditions. Cell suspensions in growth medium were prepared (2×10^6 CFU/mL), and the assays were conducted following the same mechanisms of action as reported in the previous section: (i) effect on planktonic growth; (ii) effect of biofilm formation; (iii) effect on pre-formed biofilm.

2.13. Statistical analysis

The results represent the average of three samples obtained from three independent experiments conducted on different days. Statistical analysis of the data was performed using the one-way ANOVA procedure of Statistica 6.1 (StatSoft Italy srl, Vigonza, Italy). HSD Tukey test was used to identify the differences between mean and evaluations were based on a significance level of $p \leq 0.05$.

3. Results and discussion

3.1. Fermentation kinetics and microbial characteristics

The milling by-products mixture used in this study consisted of 75 % durum wheat middling and 25 % durum wheat micronized bran, with their chemical composition provided in Table 1.

The chemical composition of the raw material employed in this study is in agreement with literature findings concerning moisture and protein content, whereas fiber content was higher in bran compared to middling (Esposito et al., 2005; Sobota et al., 2015).

The milling by-product mixture, subjected to fermentation by a selected microbial consortium containing LABs (*Latilactobacillus curvatus* LANC A, *Leuconostoc mesenteroides* LANC B and *Pediococcus pentosaceus* LANC C) and yeasts (*Kazachstania servazzii* KAZ2 and *Kazachstania unispora* FM2) (DFM), was characterized for fermentation kinetics, colour, volatile molecules profile, fatty acids profile, peptides, phytic acid, antioxidant activity, total phenols, and prebiotic activity. This characterization was then compared with an unfermented mixture (UFM) and a mixture fermented by commercial baker's yeast (BCM).

Table 2 reports the pH and acidity measured after 24 h of fermentation at 25 °C, along with the cell load of LABs and yeasts before and after the fermentation of the different samples.

Before fermentation (UFM), the pH value was 6.40, with a titratable acidity of 7.1 meqNaOH. As expected, the fermentation process led to a reduction in pH across the samples. Specifically, the DFM samples, due to the acidification activity of LABs, attained a pH of 3.95 and a titratable acidity of 30.6 meqNaOH while the BCM sample showed a pH of 5.75 and titratable acidity of 12.5 meqNaOH after 24 h.

The LABs and yeasts cell loads in the UFM samples were below the detection limit (1.0 log CFU/g). For the BCM, which was inoculated with baker's yeast, the initial yeast concentration exceeded 6 log CFU/g, reaching 8.30 log CFU/g after 24 h of fermentation. LABs presence in these samples was only observed at the end of fermentation, at a level of 1.9 log CFU/g. In the DFM samples, the initial cell count for yeasts and LABs were 4.39 and 7.08 log CFU/g, respectively, which increased to 7.87 log CFU/g for yeasts and 9.54 log CFU/g for LABs after 24 h at 25 °C. Only strains belonging to the utilized microbial consortium were detected in the DFM samples. Additionally, spontaneous fermentation was conducted, showing no significant acidification of the samples after 24 h at 25 °C, with yeasts and LABs present at levels below 3.0 log CFU/g (data not shown).

The results obtained indicate that after 24 h of fermentation, the DFM sample was characterized by a yeasts: LABs ratio of approximatively 1:100. This ratio is commonly recognized as optimal for achieving a stable wheat sourdough (Scarnato et al., 2016; Gobbetti et al., 2014; De Luca et al., 2021).

The LABs acidification activity was responsible for the low pH and high titratable acidity observed in DFM samples, which can lead to positive properties. Indeed, faster acidification leads to shorter fermentation times, potentially enhancing production efficiency and reducing associated energy costs, making it a crucial selection parameter for a sourdough starter culture intended for industrial scale application

Table 2
pH and LAB and yeast cell load of the different samples, immediately after inoculation (0 h) and at the end of the fermentation (24 h). For the same parameter, average values lacking a common letter (a-c) are significantly different.

	Acidity						Log CFU/g ± SD											
							Yeast						LAB					
	pH ± SD			meqNaOH ± SD			0 h			24 h			0 h			24 h		
UFM [1]	6.40	±	0.31[a]	7.1	±	0.4[c]	<1.0			-			<1.0			-		
DFM [2]	3.95	±	0.25[c]	30.6	±	0.7[a]	4.39	±	0.16[b]	7.87	±	0.02[a]	7.08	±	0.13	9.54	±	0.05 [a]
BCM [3]	5.75	±	0.3[b]	12.5	±	0.5[b]	6.67	±	0.02[a]	8.30	±	0.06[b]	<1.0			1.9	±	0.12[b]

[1] UFM: Unfermented milling by-product mixture.[2] DFM: Durum wheat by-products mixture fermented by microbial consortium composed of LABs and yeasts. [3] BCM: Mixture fermented by a benchmark commercial baker's yeast.

Table 3
Fat, saturated fatty acids, carbohydrates, total fibers, proteins, lactic acid and acetic acid expressed as g/100 g of dry matter (DM), of the pre-ferment DFM compared with the BCM and the UFM sample. For the same parameter, average values lacking a common letter (a-b) are significantly different.

	UFM [1]			DFM [2]			BCM [3]		
Fat g/100 g	7.2	±	0.3 [a]	7.9	±	0.4 [a]	7.2	±	0.4 [a]
Saturated fatty acids g/100 g	1.7	±	0.1 [a]	1.4	±	0.2 [a]	1.7	±	0.2 [a]
Carbohydrates g/100 g	10.9	±	0.3 [a]	10.6	±	0.5 [a]	10.2	±	0.5 [a]
Sugars g/100 g	2.1	±	0.1 [a]	0.3	±	0.1[b]	0.3	±	0.1[b]
Total fiber g/100 g	49.2	±	1.2 [a]	49.5	±	1.0 [a]	49.8	±	1.5 [a]
Protein g/100 g	21.0	±	0.3[b]	21.2	±	0.4 [ab]	21.6	±	0.3 [a]
Lactic acid g/100 g	–			0.30			0.01		
Acetic acid g/100 g	0.02			0.05			0.03		

[c1] UFM: Unfermented milling by-product mixture.[2] DFM: Durum wheat by-products mixture fermented by microbial consortium composed of LAB and yeasts. [3] BCM: Mixture fermented by a benchmark commercial bakery yeast.

(Boyaci Gunduz et al., 2022). It is generally reported that the acidification by heterofermentative LABs increases the primary activity of cereal proteases and strongly influences the proteolytic process, the activation of various enzymes and the synthesis of microbial metabolites, resulting in numerous changes during sourdough fermentation that may enhance the nutritional and functional quality of the dough and the final bread (Gänzle et al., 2008; Gobbetti et al., 2014). Moreover, acidification can decrease the phytate content of whole wheat flour due to increased activity of the flour's endogenous phytase (Leenhardt et al., 2005).

3.2. Nutritional profile and fatty acid composition

The pre-fermented samples (DFM and BCM) and the unfermented milling by-product mixture (UFM) were characterized for their chemical composition (Table 3).

Fermentation by the selected microbial consortium did not significantly affect the total and saturated fat, carbohydrate, and total fiber content compared to UFM and BCM. However, a considerable reduction in sugar content was observed in the fermented samples (DFM and BCM) compared to the unfermented one. Specifically, both DFM and BCM samples showed a sugar content of 0.34 g/100 g, while the unfermented sample contained 2.05 g/100 g of sugar. This decrease in sugar content was expected, as simple sugars serve as the primary carbon source utilized by yeast and LABs during fermentation, primarily converted into ethanol, lactic acid, and acetic acid (Li, 2004; Prückler et al., 2015). As expected, the preferment obtained by fermentation with the selected microbial consortium showed higher concentrations of lactic acid and acetic acid, reaching levels of 0.3 g/100 g and 0.05 g/100 g, respectively. In contrast, the BCM samples showed concentrations of 0.01 and 0.03 g/100 g for lactic acid and acetic acid, respectively. It is well documented that the fermentation by microbial consortia containing both homofermentative and heterofermentative LABs can facilitate rapid substrate acidification and the production of lactic acid, acetic acid and other fermentation metabolites including formic acid and ethanol (Siroli et al., 2022).

Fermentation also led to an increase in protein content, reaching values of 21.23 and 21.60 g/100 g in DFM and BCM, respectively, compared to UFM (20.96 g/100 g). However, only the sample inoculated with baker's yeast showed a significant increase in this parameter compared to the unfermented mixture.

Furthermore, not only were the total fat amounts similar across the different samples, but also the relative abundance of fatty acids. The most representative fatty acids detected in both the unfermented and fermented samples were linoleic, palmitic and oleic acids, with relative abundances of approximately 39 %, 29 % and 27 %, respectively, followed by linolenic acid (2.5 %) (data not shown). This finding aligns with the results of Narducci et al. (2019), who demonstrated that, regardless of the cultivar studied and the year of production, the distribution of the fatty acid content remained constant, with linoleic acid being the most prominent, followed by palmitic and oleic acids, linolenic acid, stearic acid, and finally palmitoleic acid.

3.3. Colour analysis

The appearance and colorimetric indices of the three samples were different, as shown in Fig. 1.

The DFM sample showed lightness (L*: 53.35 ± 1.70) and yellowness (b*: 21.44 ± 0.31) indexes similar to those of the unfermented sample (L*: 51.73 ± 0.74; b*: 20.71 ± 0.43), while its redness (a*: 6.25 ± 0.34) was significantly lower than of UFM (a*: 7.72 ± 0.25). However, DFM showed significantly higher L*, a* and b* indexes than BCM samples (L*: 50.47 ± 0.58; a*: 5.92 ± 0.07; b*: 18.27 ± 0.23).

Fermentation by the selected microbial consortium allowed to retain color stability compared to the fermentation by a commercial baker's yeast. This aspect is crucial because the color parameter affects the acceptability of the final product and the potential use of the obtained ingredients in baking. Moreover, the ability of LABs to decrease the pH and increase the presence of antioxidant substances may prevent the rapid browning of the samples. Reque et al. (2020) also confirmed the

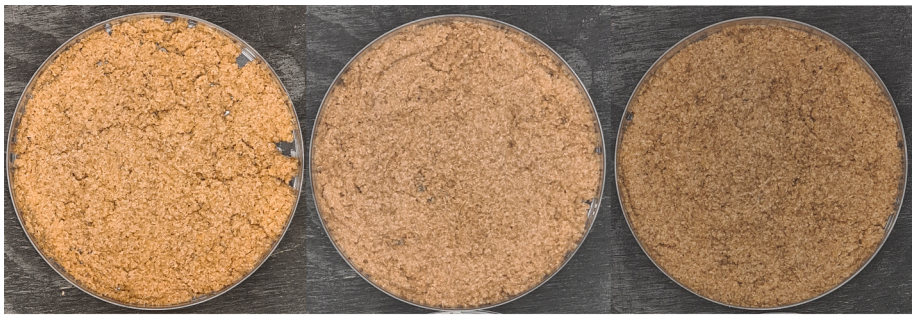

Fig. 1. Appearance of unfermented durum wheat mixture by-products (UFM) and of the pre-ferments obtained by the fermentation of the selected microbial consortia (DFM) and the baker's yeast (BCM) after 24 h of fermentation.

Table 4
Volatile compounds, expressed as ppm, detected through GC-MS-SPME in pre-ferments DFM, BCM, and the unfermented milling by-products mixture (UFM). The coefficients of variability, expressed as the percentage ratios between the standard deviations and the mean values, ranged between 2% and 5%.

		Odour Perception [a]	UFM [1] ppm eq	DFM [2]	BCM [3]
Aldehydes	Pentanal	Fruity	0.45	–	–
	Hexanal	Green, Fruity	5.38	2.61	0.22
	Heptanal	Green, Herbal	0.20	0.17	0.54
	Octanal	Waxy, Citrus, Green	–	–	0.30
	2-Heptenal, (E)-	Green	–	–	0.47
	2-Hexenal	Fruity, Green	0.30	–	–
	2-Nonenal, (E)-	Fatty, Green	0.21	0.17	0.90
	Nonanal	Waxy, Citrus	–	–	0.44
	Decanal	Sweet, Orange, Waxy	–	–	0.16
	2,4-Decadienal, (E,E)-	Fatty	–	–	0.55
	Benzaldehyde	Fruity, Sharp	0.04	0.16	0.69
Total Aldehydes			**6.58**	**3.12**	**4.27**
Ketones	3-Pentanone	Ethereal	–	0.46	–
	Methyl Isobutyl Ketone	Green, Sharp, Herbal	0.20	0.56	0.22
	2-Hexanone, 4-methyl-	Fruity, Fungal, Meaty	0.10	–	–
	3-Penten-2-one, 4-methyl-	Vegetable, Pungent	0.64	1.93	0.39
	4-Heptanone, 2,6-dimethyl-	Green, Fruity	0.14	0.21	–
	2-Heptanone	Cheese, Fruity	0.11	0.06	–
	2-Butanone, 3-hydroxy-	Buttery, Sweet, Creamy	0.09	1.46	0.34
Total ketones			**1.29**	**4.68**	**0.95**
Alcohols	1-Butanol, 3-methyl	Fruity, Sweet	–	–	7.91
	Ethyl alcohol	Ethereal	0.13	28.07	63.09
	1-Propanol	Alcoholic, Fermented	–	0.17	–
	1-Penten-3-ol	Green, Ethereal	0.47	0.25	–
	1-Nonanol	Waxy, Floral	–	–	0.95
	2-Hexanol	Winey, Fruity	0.25	0.28	–
	1-Pentanol	Fermented, Balsamic	1.37	2.03	0.95
	2-Penten-1-ol, (E)-	Green	0.45	0.49	0.19
	1-Hexanol	Herbal, Hethereal	1.91	9.40	7.86
	1-Octen-3-ol	Earthy, Mushroom	0.40	0.75	0.83
	1-Propanol, 2-methyl-	Ethereal	–	–	0.22
	Heptanol	Green, Musty	–	1.02	–
	3-Hexen-1-ol, (Z)-	Green	–	–	0.12
	1-Hexanol, 2-ethyl-	Citrus, Floral	0.12	0.22	–
	1-Octanol	Waxy, Green	0.06	1.66	0.82
	3,6-Nonadien-1-ol,	Green	–	–	0.20
	3-Nonen-1-ol, (Z)-	Waxy, Green	0.15	0.68	2.24
	Phenylethyl Alcohol	Floral	–	1.01	9.74
Total Alcohols			**5.32**	**46.01**	**95.11**
Acids	Acetic Acid	Sharp	–	4.86	2.11
	Propanoic Acid	Pungent, Acidic	–	0.07	–
	Butanoic Acid	Cheesy, Sharp	–	0.12	–
	Butanoic acid, 3-methyl-	Sweet, Mentholic	–	0.36	–
	Pentanoic Acid	Cheesy, Acidic	–	0.40	–
	Hexanoic acid	Fatty, Sour	0.27	4.95	0.95
	Heptanoic acid	Cheesy, Rancid, Sour	–	0.13	–
	Octanoic Acid	Fatty, Waxy, Rancid	–	0.72	–
Total Acids			**0.27**	**11.61**	**3.06**
Esters	Benzenacetic acid, ethyl ester	Sweet, Floral, Honey	–	–	0.40
	Benzoic acid, ethyl ester	Sweet, Wintergreen, Fruity	–	–	1.07
	Butanoic acid, ethyl ester	Fruity, Juicy	–	–	0.60
	Decanoic acid, ethyl ester	Sweet, Waxy, Fruity	–	–	0.77
	Heptanoic acid, ethyl ester	Fruity, Sweet	–	–	1.02
	Nonanoic acid, ethyl ester	Waxy, Fruity	–	–	0.71
	Octanoic acid, ethyl ester	Ester Waxy, Fruity, Winey	–	0.03	4.16
	Hexadecanoic acid, ethyl ester	Waxy, Fruity, Creamy	–	–	0.38
	Butanedioic acid, dimethyl ester	Sweet, Fruity, Green	–	–	0.35
	3-Hexanoic acid, ethyl ester	–	–	–	0.29
	Ethyl Acetate	Ethereal, Fruity, Sweet	–	8.43	16.23
	Pentanoic acid, ethyl ester	Fruity, Sweet	–	0.23	4.52
	Hexanoic acid, ethyl ester	Fruity	–	3.03	13.07
	1-Butanol, 3-methyl-, acetate	Acetate Fruity, Sweet	–	0.15	0.72
	1-Butanol, 3-methyl-, formate	Green, Fruity	–	4.18	–
Total Esters			–	**16.06**	**44.30**
Total Molecules			**13.46**	**81.48**	**147.69**

[a]Based on data reported in the literature and information found at: https://www.thegoodscentscompany.com/index.html (accessed on 25 May 2023) (The Good Scents Company Information System, 2023). [1] UFM: Unfermented milling by-product mixture.[2] DFM: Durum wheat by-products mixture fermented by microbial consortium composed of LAB and yeasts. [3] BCM: Mixture fermented by a benchmark commercial bakery yeast.

antioxidant and anti-browning properties of wheat middling fermented with *L. plantarum*, associated with the increase in total phenolic content. Conversely, fermentation by baker's yeast caused a rapid deterioration of the color parameters. This finding is consistent with Siroli et al. (2022) who observed a rapid color deterioration in a mixture of rye and wheat germ fermented with *S. cerevisiae* compared with the pre-ferment obtained by the fermentation with a microbial consortium containing LABs. The authors attributed the enhanced retention of color parameters in preferments obtained by LABs fermentation to the increased presence of antioxidant substances, such as ferulic acid.

3.4. Volatile molecule profiles

The volatile molecules profiles of the different milling by-products formulations were analysed by GC/MS/SPME immediately after formulation (UFM) and after 24 h of fermentation at 25 °C (DFM and BCM). Differences in the volatilome were observed in relation to the analysed sample. The aromatic profile of UFM, DFM and BCM contained a total of 23, 37 and 41 volatile molecules, respectively. The detected volatile molecules belonged to the chemical classes of alcohols, esters, acids, aldehydes, and ketones, as reported in Table 4.

The fermentative process increased the relative abundance and the number of the volatile molecules detected. The fermented samples showed a higher abundance of acids, alcohol and esters classes

Table 5
Peptides, total short chain fatty acids (SCFA), phenols, antioxidant activity (ABTS and DPPH) and phytic acid content in UFM, BCM and DFM. Data represent means ± SD. For the same parameter, average values lacking a common letter (a-c) are significantly different.

	UFM [1]			DFM [2]			BCM [3]		
Peptides (mg/g)	2.43	±	0.36^{b}	4.87	±	0.24 ^{a}	2.79	±	0.17^{b}
Total SCFA (mg/kg)	0.27	±	0.10^{c}	10.92	±	0.99 ^{a}	2.86	±	0.45^{b}
Total phenols (Gallic acid mg eq/kg DM)	289.7	±	13.2^{b}	396.9	±	14.5 ^{a}	297.0	±	18.0^{b}
ABTS (TROLOX mg/kg DM)	211.0	±	20.0^{b}	307.8	±	26.0 ^{a}	222.0	±	18.0^{b}
DPPH (TROLOX mg/kg DM)	158.0	±	10.0^{b}	199.0	±	14.0 ^{a}	169.0	±	8.0^{b}
Phytic acid (g/100 g DM)	2.60	±	0.09 ^{a}	1.72	±	0.06^{c}	2.34	±	0.07^{b}

[1] UFM: Unfermented milling by-product mixture.[2] DFM: Durum wheat by-products mixture fermented by microbial consortium composed of LAB and yeasts. [3] BCM: Mixture fermented by a benchmark commercial bakery yeast.

compared to the unfermented ones. This is an important aspect since the volatile molecule profile of prefermented ingredients for bakery application can be considered as crucial technological properties. Nowadays, sourdough is mainly used as an aroma improver typicality in wheat breads (Pétel et al., 2017). Specifically, the samples obtained by the fermentation with a commercial baker's yeast (BCM) were mainly characterized by the presence of alcohols and esters, with ethanol (63.09 ppm) and ethyl acetate (16.23 ppm) being the most representative molecules. These results are in accordance with Siroli et al. (2022), who observed similar trends in a mixture of rye bran and wheat germ fermented by *S. cerevisiae*. On the other hand, the strong ability of *S. cerevisiae* to produce ethanol and ethyl acetate is widely reported in literature (Saerens et al., 2010; Hazelwood et al., 2008). In contrast, the samples obtained by the fermentation with the selected microbial consortium were mainly characterized by a higher abundance of acids, such as acetic acid (4.86 ppm) and hexanoic acid (4.95 ppm), and ketones. The presence of acetic acid is typical in sourdough obtained by the fermentation with complex microbial consortia composed of LABs and yeasts (Pétel et al., 2017). Hexanoic acid results from LABs metabolism and is associated with a sour, fatty, cheesy, and sweet odour (Pétel et al., 2017).

Among esters, ethyl acetate was the most representative molecule in both the preferments, mainly in BCM, while it was not detected in UFM. The presence of esters in sourdough mainly derives from yeasts metabolism during flour fermentation, and the highest content of esters in BCM is related to the activity of *Saccharomyces cerevisiae,* known as a high ester producer (Jin et al., 2021; Pico et al., 2015; Zhang et al., 2018). Regarding alcohols, ethanol was the most represented one in both the pre-fermented samples, but the content in samples obtained with commercial baker's yeast was significantly higher than in the sample inoculated with the selected microbial consortium. This is due to the alcohol dehydrogenase activity of *Saccharomyces cerevisiae,* making it one of the most important microorganisms from a technological point of view (de Smidt et al., 2008). Instead, 1-hexanol, characterizing the DFM sample, is a lipid oxidation compound mainly produced by LABs (Gobbetti et al., 1995; Kaseleht et al., 2011). In fact, some LABs can convert certain lipid oxidation compounds into their corresponding alcohols (Vermeulen et al., 2007) and significantly reduce lipid oxidation during fermentation (Czerny & Schieberle, 2002; Gänzle et al., 2007).

3.5. Functionality

The samples were characterized in terms of peptides, total short chain fatty acids (SCFA), phenol content, antioxidant activity (ABTS and DPPH), and phytic acid content and the results are reported in Table 5.

The OPA analysis showed that the fermentation by the selected consortium of LABs and yeasts (DFM) significantly increased the peptide content (4.87 mg/g) compared to UFM (2.47 mg/g) and BCM (2.79 mg/g) samples. The results are in agreement with those reported by Siroli et al. (2022), who demonstrated the ability of a microbial consortium composed of *Latilactobacillus curvatus*, *Kazachstania unispora* and *Kazachstania servazii* to significantly increase the peptide content of a mixture of rye bran and germ after 24 h of fermentation. These findings were also confirmed by the SDS-PAGE analysis, which showed a reduction of high molecular weight peptides in the DFM samples compared to the BCM ones (data not showed). The high peptide content in the DFM samples may be attributed to the proteolytic activity of the microbial consortium used in the trial. Indeed, the LABs used in this study, *Latilactobacillus curvatus*, *Leuconostoc mesenteroides* and *Pediococcus pentosaceus,* are characterized by strong proteolytic activity (Zotta et al., 2006; Mamhoud et al., 2016; Bartkiene et al.,2017). The increased peptide content of wheat bran fractions fermented by a consortium composed by *Lactobacillus brevis* and *Kazachstania exigua* was also reported by Coda et al. (2014), who highlighted that microbial proteolysis was mainly due to the activity of LABs. Additionally, the proteolytic activity of LABs and their ability to increase the content of bioactive peptides in various fermented foods, such as milk and dairy products, spinach, lentil, cereals, soy, corn, rice and amaranth, are well documented (Scarnato et al., 2016; Tagliazucchi et al., 2019; Pessione and Cirrincione, 2016). However, *Kazachstania* spp. are also characterized by proteolytic activity and may contributes to the increased availability of peptides (Palla et al., 2017).

The fermentation carried out by the selected microbial consortium caused a significant increase in SCFAs compared to UFM and BCM samples. In fact, the DFM samples were characterized by a SCFAs content of 10.92 mg/kg, while the UFM and BCM samples contained 0.27 and 2.86 mg/kg of SCFAs, respectively. Overall, the fermentation process led to an increase in the SCFAs content, but the presence of LABs significantly increased the SCFAs content in DFM samples. Pérez-Alvarado et al. (2022) highlighted that the strong metabolic activity of LABs in sourdough and fermented bran increased the SCFAs content. The SCFAs detected in DFM samples were acetic acid, propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid and octanoic acid. However, the dominant SCFAs were acetic acid and hexanoic acid. The increase in SCFAs content is very important as their health benefits are widely reported in literature (Xiong et al., 2022; Tan et al., 2014). In fact, SCFAs are easily absorbed by the host with systemic roles in insulin secretion, inflammation, lipid metabolism, and beneficial effects on intestinal function (Kasubuchi et al., 2015; McLoughlin et al., 2017). In addition, SCFAs contribute to the production of bread crumb flavours during baking (Birch et al., 2013).

The DFM samples also exhibited a significant increase in total phenolic compounds. Specifically, the total phenol content of the DFM samples was significantly higher than that in UFM and BCM samples. This expected result can be attributed to the enzymatic activities of the strains applied in the consortium, which led to the release of phenolic compounds. Cereal bran is indeed very rich in phenolic compounds; however, they are bound to the cell wall matrix and are therefore not readily available (Adebo et al., 2020; Moore et al., 2007). Fermentation with selected microbial consortia could release the insoluble bounded phenolic acids, thereby increasing their availability and enhancing the protective and physiological functions these compounds can exert on human health. Among the benefits of phenolic compounds, the most notable are the antioxidant properties and, consequently, their ability to

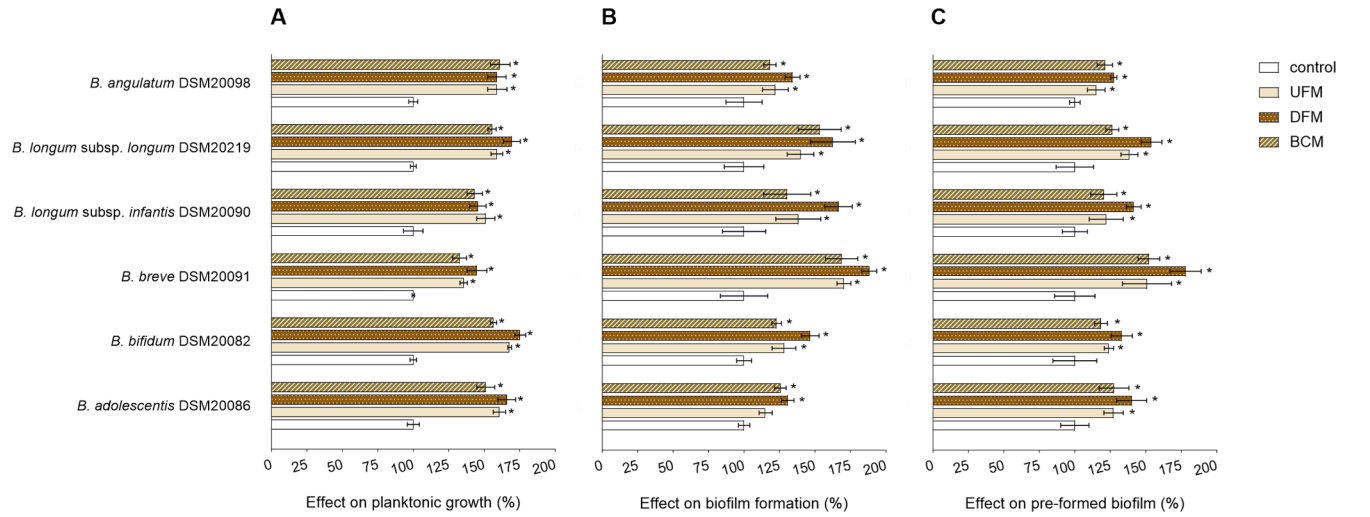

Fig. 2. Effects of milling by products (UFM, DFM and BCM) on planktonic growth (A), biofilm formation (B), and pre-formed biofilms (C) of *Bifidobacterium* spp. Results are expressed as percentages with respect to control (100 %) (means ± SD, $n = 3$). * $p < 0.05$.

protect against degenerative diseases, such as cancer and heart disease, where reactive oxygen species are implicated (superoxide anions, hydroxyl radicals, peroxyl radicals) (Saura-Calixto, 2011).

As expected, the high concentration of phenolic compounds in the DFM samples resulted in significantly higher antioxidant activity compared to the UFM and BCM samples. The antioxidant capacity of the samples was assessed using ABTS and DPPH assays, both of which exhibited the same trend observed in determination of total phenol content. These findings are in agreement with those of Siroli et al. (2022), who observed a substantial increase in phenolic compounds and antioxidant activity in a preferment of rye bran and wheat germ fermented by a microbial consortium composed of *L. curvatus* and two strains of *Kazachstania spp*. These results were attributed to the LABs ability to promote the synthesis of exopolysaccharides, biomolecules with antioxidant activity, and glutathione, the main non-enzymatic antioxidant and scavenger of free-radicals (Laurent-Babot & Guyot, 2017). Several authors reported the ability of LABs to enhance the antioxidant activity of wheat sourdough. For instance, Pejcz et al. (2021) demonstrated that *Lactiplantibacillus plantarum* and *Lacticaseibacillus casei*, used individually in sourdough production, increased antioxidant activity by boosting the amount of easily extractable phenolic compounds. Moreover, Abedfar et al., 2018 showed the ability of *P. pentosaceus* to produce antioxidant exopolysaccharides in sourdough. The combined effect of LABs and yeasts could have also contributed to an increase in antioxidant compounds. In fact, Coda et al. (2014) observed heightened antioxidant activity and peptide content in a wheat bran sourdough obtained through fermentation by *Lb. brevis* and *Kazachstania exigua.*

Finally, a decrease in phytic acid was observed as a result of the fermentation process compared with unfermented mixture. In particular, DFM samples fermented by the selected microbial consortium exhibited a higher reduction of phytic acid compared to BCM samples fermented by commercial baker's yeast. The ability of the selected microbial consortia to reduce phytate content is an important achievement and aligns with results reported in the literature which demonstrate a reduced content of phytic acid in wheat bran and germ fermented by LABs (Zhao et al., 2017; Spaggiari et al., 2020; Siroli et al., 2022). The degradation of phytic acid is generally attributed to the phytase activity of LABs, which, aided by acidification and the consequent reduction of pH, can promote the activation of endogenous phytases (Rizzello et al., 2010; Siroli et al., 2022).

This finding is particularly significant as phytic acid is an anti-nutritional compound capable of interfering with the absorption of essential micronutrients such as iron, zinc, calcium, magnesium and manganese due to its chelating properties (Bohn et al., 2004; Gupta et al., 2015; Phillippy, 2006). The decrease of phytic acid improves the bioavailability of various cations, thereby enhancing functionality and nutritional value of pre-fermented ingredients (Coulibaly et al., 2011; Gupta et al., 2015).

3.6. Prebiotic activity

The pre-ferments from the durum wheat by-products mixture, fermented with the selected microbial consortium (DFM), were initially sought for their ability to stimulate the planktonic growth of *Bifidobacterium* species highly represented in human colon, i.e., *B. adolescentis*, *B. bifidum*, *B. breve*, *B. longum* subsp. *longum*, *B. longum* subsp. *infantis* and *B. angulatum* (Martin et al., 2016). For comparison, pre-ferments obtained with commercial baker's yeast (BCM) and the unfermented mixtures (UFM) were also tested. Results are reported in Fig. 2A. All samples significantly improved the growth of *Bifidobacterium* spp., with growth percentages ranging from 145 to 175 %, 133–161 % and 136–168 % in the presence of DFM, BCM and UFM, respectively. The bifidogenic activity observed for DFM samples exceeded that of the unfermented mixtures for two strains out of six ($p < 0.05$), while for the remaining bifidobacteria strains, the activities of DFM and UFM samples were comparable ($p > 0.05$). Furthermore, DFM were more effective than BCM samples in stimulating the growth of four bifidobacteria out of six ($p < 0.05$), suggesting that the fermentation of wheat bran and germ by LABs can increase the prebiotic potential of pre-ferments.

Since microorganisms are primarily present in their microenvironment as adherent rather than as free-floating cells (Motta et al., 2021), the bifidogenic potential was further investigated in terms of ability to stimulate *Bifidobacterium* spp. biofilms. The data clearly revealed that both pre-ferments and unfermented mixtures were able to promote the formation of *Bifidobacterium* spp. biofilms (Fig. 2B) and to stimulate the pre-formed ones (Fig. 2C). Specifically, DFM samples were more efficient than the unfermented mixtures in promoting the formation of biofilms for five out of six bifidobacteria strains and in stimulating pre-formed biofilm for three bifidobacteria ($p < 0.05$), reaching biofilm formation percentages of 131–188 % and 126–178 %, respectively. Consistent with what was observed for planktonic cultures, in half of the cases, the stimulating activity attributed to DFM samples was also significantly higher than that of BCM samples. Indeed, the fermentation of wheat bran and germ by LABs may lead to the production of metabolites with prebiotic behaviour, including oligosaccharides and exopolysaccharides (Poutanen et al., 2009). In particular, the latter have been reported to selectively increase the growth of probiotic species

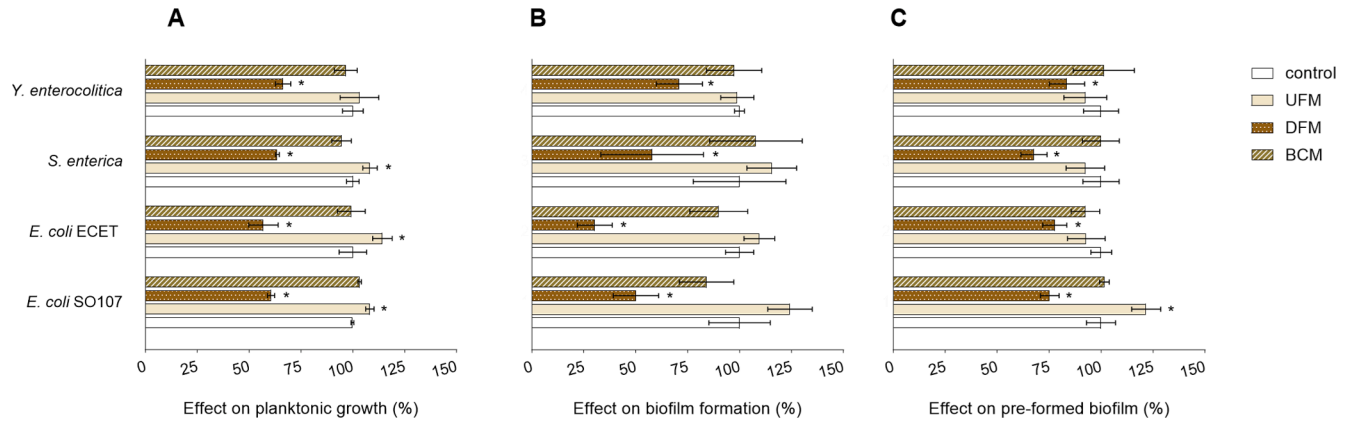

Fig. 3. Effects of milling by products (UFM, DFM and BCM) on planktonic growth (A), biofilm formation (B), and pre-formed biofilms (C) of gastrointestinal pathogens. Results are expressed as percentages with respect to control (100 %) (means ± SD, $n = 3$). * $p < 0.05$.

(*Bifidobacterium* spp. and *Lactobacillus* spp.) and to act as biofilm-promoting molecules (Giordani et al., 2023). This aspect is of particular importance as the capability to stimulate the biofilms of *Bifidobacterium* spp. can, in turn, favour a strong colonization of such bacteria in the intestinal tract, thus improving their health-promoting activity (Kelly et al., 2020).

3.7. Antimicrobial activity against gastrointestinal pathogens

The impact of pre-ferments and unfermented mixtures on opportunistic (*E. coli* SO107) and virulent (*E. coli* ECET, *S. enterica* and *Y. enterocolitica*) gastrointestinal pathogens was also investigated to exclude possible undesired stimulating effects. Results on planktonic cultures, biofilm formation and pre-formed biofilms of pathogenic strains are depicted in Fig. 3A, 3B and 3C, respectively. The unfermented mixtures slightly promoted the proliferation of *E. coli* strains and *S. enterica* (+8–14 %, $p < 0.05$); no effects were observed on the biofilms of virulent strains, while UFM samples enhanced the pre-formed biofilm of *E. coli* by 22 %.

While the mixtures fermented by baker's yeast did not impact on pathogens grown as free-floating form or as biofilms, the mixtures fermented by LABs and yeasts reduced the growth of all pathogens by 34–43 % and exerted a significant anti-biofilm activity, both in terms of inhibiting pathogens' biofilm formation (inhibition of 30–70 %) and dispersing pre-formed biofilms (eradication of 17–32 %). This finding is consistent with Siroli et al. (2022) and can be attributed to the lower pH of the DFM samples and the presence of metabolites with antibacterial activity, such as bioactive peptides, SCFA and phenols. These and other LABs-derived metabolites, including exopolysaccharides and biosurfactants, can also exert anti-biofilm activities against non-probiotic bacteria (Giordani et al., 2023, Hussaini et al., 2023). This aspect, together with the prebiotic activity, can be important to avoid the formation by enteropathogens of strong and difficult to treat biofilms on gastrointestinal mucosa.

4. Conclusion

This study demonstrated how sourdough fermentation with selected microbial consortia of lactic acid bacteria and yeast represents a strategy for improving the overall characteristics of by-products of the milling industry. Specifically, the prefermented ingredient obtained through the fermentation of durum wheat bran was characterized by a volatile molecules profile that includes compounds associated with a positive odor perception, typical of traditional sourdough used in bakery applications. Additionally, the preferments obtained by the fermentation of the selected microbial consortium exhibited a significant increase, compared to the references, in functional properties including SCFA, antioxidant activity, total phenol and peptide content. This increase was associated with a reduction of antinutritional compounds such as phytic acid. Furthermore, the innovative prefermented ingredient inhibited the growth and exerted a significant anti-biofilm activity against different gastrointestinal pathogens, while also stimulating the growth of probiotic microorganisms. In conclusion, the preferment obtained in this work displayed interesting properties that may promote its use as food ingredients capable of imparting positive sensorial and functional properties to bread and semolina-pasta. However, future studies should focus on assessing the overall properties of bakery products obtained by including these ingredients into the recipe.

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CRediT authorship contribution statement

Samantha Rossi: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Davide Gottardi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation. **Lorenzo Siroli:** Writing – review & editing, Writing – original draft, Software, Data curation, Conceptualization. **Barbara Giordani:** Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis. **Beatrice Vitali:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Lucia Vannini:** Visualization, Conceptualization. **Francesca Patrignani:** Writing – review & editing, Conceptualization. **Rosalba Lanciotti:** Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

AACC. *Approved Methods of the AACC, 10th ed.*; American Association of Cereal Chemists: St. Paul, MN, USA, (2000). Retrieved from http://www.sciepub.com/reference/241265. Accessed November 11, 2022.

Abedfar, A., Hosseininezhad, M., Sadeghi, A., Raeisi, M., & Feizy, J. (2018). Investigation on "spontaneous fermentation" and the productivity of microbial exopolysaccharides by *Lactobacillus plantarum* and *Pediococcus pentosaceus* isolated from wheat bran sourdough. *LWT-Food Science and Technology, 96*, 686–693. https://doi.org/10.1016/j.lwt.2018.05.071

Alzuwaid, N. T., Pleming, D., Fellows, C. M., Laddomada, B., & Sissons, M. (2021). Influence of durum wheat bran particle size on phytochemical content and on leavened bread baking quality. *Foods, 10*(3), 489. https://doi.org/10.3390/foods10030489

Aoac 920.39 2003_05 *Extracto Etereo Dietileter-VSIP. INFO*. Retrieved from https://vsip.info/aoac-92039-200305-extractoetereo-dietileter-2-pdf-free.html. Accessed November 11, 2022.

AOAC 986.11-1988, *Phytate in Foods Anion Exchange Method*-$14.15: AOAC Official Method. Retrieved from http://www.aoacofficialmethod.org/index.php?main_page=product_info&cPath=1&products_id=313. Accessed November 11, 2022.

Arte, E., Rizzello, C. G., Verni, M., Nordlund, E., Katina, K., & Coda, R. (2015). Impact of enzymatic and microbial bioprocessing on protein modification and nutritional properties of wheat bran. *Journal of Agricultural and Food Chemistry, 63*(39), 8685–8693. https://doi.org/10.1021/acs.jafc.5b03495

Bartkiene, E., Bartkevics, V., Krungleviciute, V., Pugajeva, I., Zadeike, D., & Juodeikiene, G. (2017). Lactic acid bacteria combinations for wheat sourdough preparation and their influence on wheat bread quality and acrylamide formation. *Journal of food science, 82*(10), 2371–2378. https://doi.org/10.1111/1750-3841.13858

Bartkiene, E., Mozuriene, E., Lele, V., Zokaityte, E., Gruzauskas, R., Jakobsone, I., & Bartkevics, V. (2020). Changes of bioactive compounds in barley industry by-products during submerged and solid state fermentation with antimicrobial Pediococcus acidilactici strain LUHS29. *Food Science & Nutrition, 8*(1), 340–350. https://doi.org/10.1002/fsn3.1311

Bertsch, A., Roy, D., & LaPointe, G. (2020). Fermentation of wheat bran and whey permeate by mono-cultures of Lacticaseibacillus rhamnosus strains and co-culture with yeast enhances bioactive properties. *Frontiers in Bioengineering and Biotechnology, 8*, 956. https://doi.org/10.3389/fbioe.2020.00956

Birch, A. N., Petersen, M. A., & Hansen, Å. S. (2013). The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature. *LWT-Food Science and Technology, 50*(2), 480–488. https://doi.org/10.1016/j.lwt.2012.08.019

Bohn, T., Davidsson, L., Walczyk, T., & Hurrell, R. F. (2004). Phytic acid added to white-wheat bread inhibits fractional apparent magnesium absorption in humans. *The American Journal of Clinical Nutrition, 79*(3), 418–423. https://doi.org/10.1093/AJCN/79.3.418

Boselli, E., Velazco, V., Caboni, M. F., & Lercker, G. (2001). Pressurized liquid extraction of lipids for the determination of oxysterols in egg-containing food. *Journal of Chromatography A, 917*(1–2), 239–244. https://doi.org/10.1016/S0021-9673(01)00688-4

Buddrick, O., Jones, O. A., Cornell, H. J., & Small, D. M. (2014). The influence of fermentation processes and cereal grains in wholegrain bread on reducing phytate content. *Journal of Cereal Science, 59*(1), 3–8. https://doi.org/10.1016/j.jcs.2013.11.006

Burns, P., Patrignani, F., Serrazanetti, D., Vinderola, G. C., Reinheimer, J. A., Lanciotti, R., & Guerzoni, M. E. (2008). Probiotic Crescenza cheese containing *Lactobacillus casei* and *Lactobacillus acidophilus* manufactured with high-pressure homogenized milk. *Journal of Dairy Science, 91*(2), 500–512. https://doi.org/10.3168/jds.2007-0516

Cai, L., Choi, I., Hyun, J. N., Jeong, Y. K., & Baik, B. K. (2014). Influence of bran particle size on bread-baking quality of whole grain wheat flour and starch retrogradation. *Cereal Chemistry, 91*(1), 65–71. https://doi.org/10.1094/CCHEM-02-13-0026-R

Cardenia, V., Sgarzi, F., Mandrioli, M., Tribuzio, G., Rodriguez-Estrada, M. T., & Toschi, T. G. (2018). Durum wheat bran by-products: Oil and phenolic acids to be valorized by industrial symbiosis. *European Journal of Lipid Science and Technology, 120*(8), 1700209. https://doi.org/10.1002/ejlt.201700209

Cheng, W., Sun, Y., Fan, M., Li, Y., Wang, L., & Qian, H. (2022). Wheat bran, as the resource of dietary fiber: A review. *Critical reviews in food science and nutrition, 62*(26), 7269–7281. https://doi.org/10.1080/10408398.2021.1913399

Coda, R., Katina, K., & Rizzello, C. G. (2015). Bran bioprocessing for enhanced functional properties. *Current Opinion in Food Science, 1*, 50–55. https://doi.org/10.1016/j.cofs.2014.11.007

Coda, R., Rizzello, C. G., Curiel, J. A., Poutanen, K., & Katina, K. (2014). Effect of bioprocessing and particle size on the nutritional properties of wheat bran fractions. *Innovative Food Science & Emerging Technologies, 25*, 19–27. https://doi.org/10.1016/j.ifset.2013.11.012

Coda, R., Rizzello, C. G., Pinto, D., & Gobbetti, M. (2012). Selected lactic acid bacteria synthesize antioxidant peptides during sourdough fermentation of cereal flours. *Applied and Environmental Microbiology, 78*(4), 1087–1096. https://doi.org/10.1128/AEM.06837-11

Costantini, L., Lukšič, L., Molinari, R., Kreft, I., Bonafaccia, G., Manzi, L., & Merendino, N. (2014). Development of gluten-free bread using tartary buckwheat and chia flour rich in flavonoids and omega-3 fatty acids as ingredients. *Food Chemistry, 165*, 232–240. https://doi.org/10.1016/j.foodchem.2014.05.095

Coulibaly, A., Kouakou, B., & Chen, J. (2011). Phytic acid in cereal grains: Structure, healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional quality. *American Journal of Plant Nutrition and Fertilization Technology, 1*(1), 1–22. https://doi.org/10.3923/AJPNFT.2011.1.22

Czerny, M., & Schieberle, P. (2002). Important aroma compounds in freshly ground wholemeal and white wheat flour identification and quantitative changes during sourdough fermentation. *Journal of Agricultural and Food Chemistry, 50*(23), 6835–6840. https://doi.org/10.1021/JF020638P

da Rocha Lemos Mendes, G., Souto Rodrigues, P., de las Mercedes Salas-Mellado, M., Fernandes de Medeiros Burkert, J., & Badiale-Furlong, E., 2021. Defatted rice bran as a potential raw material to improve the nutritional and functional quality of cakes. Plant Foods for Human Nutrition, 76, 46-52. Doi: 10.1007/s11130-020-00872-6.

De Luca, L., Aiello, A., Pizzolongo, F., Blaiotta, G., Aponte, M., & Romano, R. (2021). Volatile organic compounds in breads prepared with different sourdoughs. *Applied Sciences, 11*(3), 1330. https://doi.org/10.3390/app11031330

De Munter, J. S. L., Hu, F. B., Spiegelman, D., Franz, M., & Van Dam, R. M. (2007). Whole grain, bran, and germ intake and risk of type 2 diabetes: A prospective cohort study and systematic review. *pLoS medicine, 4*(8), e261.

de Smidt, O., du Preez, J. C., & Albertyn, J. (2008). The alcohol dehydrogenases of *Saccharomyces cerevisiae*: A comprehensive review. *FEMS Yeast Research, 8*(7), 967–978. https://doi.org/10.1111/J.1567-1364.2008.00387.X

de Sousa, T., Ribeiro, M., Sabença, C., & Igrejas, G. (2021). The 10,000-year success story of wheat! *Foods, 10*(9), 2124. https://doi.org/10.3390/foods10092124

dos Santos Oliveira, M., Feddern, V., Kupski, L., Cipolatti, E. P., Badiale-Furlong, E., & de Souza-Soares, L. A. (2011). Changes in lipid, fatty acids and phospholipids composition of whole rice bran after solid-state fungal fermentation. *Bioresource Technology, 102*(17), 8335–8338. https://doi.org/10.1016/j.biortech.2011.06.025

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2010). Scientific Opinion on the substantiation of health claims related to wheat bran fibre and increase in faecal bulk (ID 3066), reduction in intestinal transit time (ID 828, 839, 3067, 4699) and contribution to the maintenance or achievement of a normal body weight (ID 829) pursuant to Article 13 (1) of Regulation (EC) No 1924/2006. *EFSA Journal, 8(10),* 1817.

Esposito, F., Arlotti, G., Bonifati, A. M., Napolitano, A., Vitale, D., & Fogliano, V. (2005). Antioxidant activity and dietary fibre in durum wheat bran by-products. *Food Research International, 38*(10), 1167–1173. https://doi.org/10.1016/j.foodres.2005.05.002

Galanakis, C. M. (2022). Sustainable applications for the valorization of cereal processing by-products. *Foods, 11*(2), 241. https://doi.org/10.3390/foods11020241

Gänzle, M. G., Loponen, J., & Gobbetti, M. (2008). Proteolysis in sourdough fermentations: Mechanisms and potential for improved bread quality. *Trends in Food Science & Technology, 19*(10), 513–521. https://doi.org/10.1016/J.TIFS.2008.04.002

Gänzle, M. G., Vermeulen, N., & Vogel, R. F. (2007). Carbohydrate, peptide and lipid metabolism of lactic acid bacteria in sourdough. *Food Microbiology, 24*(2), 128–138. https://doi.org/10.1016/J.FM.2006.07.006

Giordani, B., Naldi, M., Croatti, V., Parolin, C., Erdoğan, Ü., Bartolini, M., & Vitali, B. (2023). Exopolysaccharides from vaginal lactobacilli modulate microbial biofilms. *Microbial Cell Factories, 22*(1), 45. https://doi.org/10.1186/s12934-023-02053-x

Giordani, B., Parolin, C., Abruzzo, A., Foschi, C., Marangoni, A., Luppi, B., & Vitali, B. (2023). *Limosilactobacillus vaginalis* exerts bifidogenic effects: A novel postbiotic strategy for infant prebiotic supplementation. *Nutrients, 15*(20), 4433. https://doi.org/10.3390/nu15204433

Gobbetti, M., Rizzello, C. G., Di Cagno, R., & De Angelis, M. (2014). How the sourdough may affect the functional features of leavened baked goods. *Food microbiology, 37*, 30–40. https://doi.org/10.1016/j.fm.2013.04.012

Gobbetti, M., Simonetti, M. S., Corsetti, A., Santinelli, F., Rossi, J., & Damiani, P. (1995). Volatile compound and organic acid productions by mixed wheat sour dough starters: Influence of 356 fermentation parameters and dynamics during baking. *Food Microbiology, 12*(C), 497–507. https://doi.org/10.1016/S0740-0020(95)80134-0

Gottardi, D., Siroli, L., Braschi, G., Rossi, S., Bains, N., Vannini, L., & Lanciotti, R. (2023). Selection of yarrowia lipolytica strains as possible solution to valorize untreated cheese whey. *Fermentation, 9*(1), 51. https://doi.org/10.3390/fermentation9010051

Boyaci Gunduz, C. P., Agirman, B., Gaglio, R., Franciosi, E., Francesca, N., Settanni, L., & Erten, H. (2022). Evaluation of the variations in chemical and microbiological properties of the sourdoughs produced with selected lactic acid bacteria strains during fermentation. *Food Chemistry: X, 14*, Article 100357. https://doi.org/10.1016/j.fochx.2022.100357

Gupta, R. K., Gangoliya, S. S., & Singh, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology, 52*(2), 676–684. https://doi.org/10.1007/S13197-013-0978-Y/FIGURES/1

Hazelwood, L. A., Daran, J. M., Van Maris, A. J., Pronk, J. T., & Dickinson, J. R. (2008). The Ehrlich pathway for fusel alcohol production: A century of research on Saccharomyces cerevisiae metabolism. *Applied and environmental microbiology, 74*(8), 2259–2266. https://doi.org/10.1128/AEM.02625-07

Hussaini, I. M., Oyewole, O. A., Sulaiman, M. A., Dabban, A. I., Sulaiman, A. N., & Tarek, R. (2023). Microbial anti-biofilms: Types and mechanism of action. *Research in Microbiology, 104111*. https://doi.org/10.1016/j.resmic.2023.104111

Jin, J., Nguyen, T. T. H., Humayun, S., Park, S. H., Oh, H., Lim, S., Mok, I. K., Li, Y., Pal, K., & Kim, D. (2021). Characteristics of sourdough bread fermented with

Pediococcus pentosaceus and *Saccharomyces cerevisiae* and its bio-preservative effect against Aspergillus flavus. *Food Chemistry, 345*, Article 128787. https://doi.org/10.1016/J.FOODCHEM.2020.128787

Kaseleht, K., Paalme, T., Mihhalevski, A., & Sarand, I. (2011). Analysis of volatile compounds produced by different species of lactobacilli in rye sourdough using multiple headspace extraction. *International Journal of Food Science & Technology, 46*(9), 1940–1946. https://doi.org/10.1111/J.1365-2621.2011.02705.X

Kasubuchi, M., Hasegawa, S., Hiramatsu, T., Ichimura, A., & Kimura, I. (2015). Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients, 7*(4), 2839–2849. https://doi.org/10.3390/nu7042839

Katina, K., Juvonen, R., Laitila, A., Flander, L., Nordlund, E., Kariluoto, S., & Poutanen, K. (2012). Fermented wheat bran as a functional ingredient in baking. *Cereal chemistry, 89*(2), 126–134. https://doi.org/10.1094/CCHEM-08-11-0106

Kelly, S. M., Lanigan, N., O'Neill, I. J., Bottacini, F., Lugli, G. A., Viappiani, A., & van Sinderen, D. (2020). Bifidobacterial biofilm formation is a multifactorial adaptive phenomenon in response to bile exposure. *Scientific reports, 10*(1), 11598. https://doi.org/10.1038/s41598-020-68179-9

Khalid, K. H., Ohm, J. B., & Simsek, S. (2017). Whole wheat bread: Effect of bran fractions on dough and end-product quality. *Journal of Cereal Science, 78*, 48–56. https://doi.org/10.1016/j.jcs.2017.03.011

Laurent-Babot, C., & Guyot, J. P. (2017). Should research on the nutritional potential and health benefits of fermented cereals focus more on the general health status of populations in developing countries? *Microorganisms, 5*(3), 40. https://doi.org/10.3390/MICROORGANISMS5030040

Leenhardt, F., Levrat-Verny, M. A., Chanliaud, E., & Rémésy, C. (2005). Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. *Journal of Agricultural and Food Chemistry, 53*(1), 98–102. https://doi.org/10.1021/JF049193Q/ASSET/IMAGES/LARGE/JF049193QF00008.JPEG

Li, K. Y. (2004). Fermentation: Principles and microorganisms. In *Handbook of food and beverage fermentation technology* (pp. 706–722). CRC Press.

Luchese, C. L., Gurak, P. D., & Marczak, L. D. F. (2015). Osmotic dehydration of physalis (Physalis peruviana L.): Evaluation of water loss and sucrose incorporation and the quantification of carotenoids. LWT-Food. *Science and Technology, 63*(2), 1128–1136. https://doi.org/10.1016/j.lwt.2015.04.060

Luithui, Y., Baghya Nisha, R., & Meera, M. S. (2019). Cereal by-products as an important functional ingredient: Effect of processing. *Journal of Food Science and Technology, 56*, 1–11. https://doi.org/10.1007/s13197-018-3461-y

Mamhoud, A., Nionelli, L., Bouzaine, T., Hamdi, M., Gobbetti, M., & Rizzello, C. G. (2016). Selection of lactic acid bacteria isolated from Tunisian cereals and exploitation of the use as starters for sourdough fermentation. *International Journal of Food Microbiology, 225*, 9–19. https://doi.org/10.1016/j.ijfoodmicro.2016.03.004

Manini, F., Brasca, M., Plumed-Ferrer, C., Morandi, S., Erba, D., & Casiraghi, M. C. (2014). Study of the chemical changes and evolution of microbiota during sourdoughlike fermentation of wheat bran. *Cereal Chemistry, 91*(4), 342–349. https://doi.org/10.1094/CCHEM-09-13-0190-CESI

Marco, C., Pérez, G., Ribotta, P., & Rosell, C. M. (2007). Effect of microbial transglutaminase on the protein fractions of rice, pea and their blends. *Journal of the Science of Food and Agriculture, 87*(14), 2576–2582. https://doi.org/10.1002/jsfa.3006

Martin, R., Makino, H., Cetinyurek Yavuz, A., Ben-Amor, K., Roelofs, I Ishikawa, E., ... & Knol, J. (2016). Early-life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. *PloS one, 11(6),* e0158498. Doi: 10.1371/journal.pone.0158498.

McLoughlin, R. F., Berthon, B. S., Jensen, M. E., Baines, K. J., & Wood, L. G. (2017). Short-chain fatty acids, prebiotics, synbiotics, and systemic inflammation: A systematic review and meta-analysis. *The American Journal of Clinical Nutrition, 106*(3), 930–945. https://doi.org/10.3945/ajcn.117.156265

Miller, N. J., & Rice-Evans, C. A. (1997). Factors influencing the antioxidant activity determined by the ABTS•+ radical cation assay. *Free Radical Research, 26*(3), 195–199. https://doi.org/10.3109/10715769709097799

Moore, J., Cheng, Z., Hao, J., Guo, G., Liu, J. G., Lin, C., & Yu, L. (2007). Effects of solid-state yeast treatment on the antioxidant properties and protein and fiber compositions of common hard wheat bran. *Journal of Agricultural and Food Chemistry, 55*(25), 10173–10182. https://doi.org/10.1021/jf071590o

Motta, J. P., Wallace, J. L., Buret, A. G., Deraison, C., & Vergnolle, N. (2021). Gastrointestinal biofilms in health and disease. *Nature Reviews Gastroenterology & Hepatology, 18*, 4–334. https://doi.org/10.1038/s41575-020-00397-y

Narducci, V., Finotti, E., Galli, V., & Carcea, M. (2019). Lipids and fatty acids in Italian durum wheat (Triticum durum Desf.) cultivars. *Foods, 8*(6), 223. https://doi.org/10.3390/foods8060223

Nayik, G. A., Tufail, T., Anjum, F. M., & Ansari, M. J. (Eds.). (2023). *Cereal Grains: Composition, Nutritional Attributes, and Potential Applications*. CRC Press.

Palla, M., Cristani, C., Giovannetti, M., & Agnolucci, M. (2017). Identification and characterization of lactic acid bacteria and yeasts of PDO Tuscan bread sourdough by culture dependent and independent methods. *International Journal of Food Microbiology, 250*, 19–26. https://doi.org/10.1016/j.ijfoodmicro.2017.03.015

Pasqualone, A., Laddomada, B., Centomani, I., Paradiso, V. M., Minervini, D., Caponio, F., & Summo, C. (2017). Bread making aptitude of mixtures of re-milled semolina and selected durum wheat milling by-products. *LWT-Food Science and Technology, 78*, 151–159. https://doi.org/10.1016/j.lwt.2016.12.032

Pejcz, E., Lachowicz-Wiśniewska, S., Nowicka, P., Wojciechowicz-Budzisz, A., Spychaj, R., & Gil, Z. (2021). Effect of inoculated lactic acid fermentation on the fermentable saccharides and polyols, polyphenols and antioxidant activity changes in wheat sourdough. *Molecules, 26*(14), 4193. https://doi.org/10.3390/molecules26144193

Pérez-Alvarado, O., Zepeda-Hernández, A., Garcia-Amezquita, L. E., Requena, T., Vinderola, G., & García-Cayuela, T. (2022). Role of lactic acid bacteria and yeasts in sourdough fermentation during breadmaking: Evaluation of postbiotic-like components and health benefits. *Frontiers in Microbiology, 13*, Article 969460. https://doi.org/10.3389/fmicb.2022.969460

Pessione, E., & Cirrincione, S. (2016). Bioactive molecules released in food by lactic acid bacteria: Encrypted peptides and biogenic amines. *Frontiers in microbiology, 7*, 876. https://doi.org/10.3389/fmicb.2016.00876

Pétel, C., Onno, B., & Prost, C. (2017). Sourdough volatile compounds and their contribution to bread: A review. *Trends in Food Science & Technology, 59*, 105–123. https://doi.org/10.1016/j.tifs.2016.10.015

Phillippy, B. Q. (2006). Transport of calcium across Caco-2 cells in the presence of inositol hexakisphosphate. *Nutrition Research, 26*(3), 146–149. https://doi.org/10.1016/J.NUTRES.2006.02.008

Pico, J., Bernal, J., & Gómez, M. (2015). Wheat bread aroma compounds in crumb and crust: A review. *Food Research International, 75*, 200–215. https://doi.org/10.1016/J.FOODRES.2015.05.051

Pointer, M. R. (1981). A comparison of the CIE 1976 colour spaces. *Color Research & Application, 6*(2), 108–118. https://doi.org/10.1002/col.5080060212

Pontonio, E., Lorusso, A., Gobbetti, M., & Rizzello, C. G. (2017). Use of fermented milling by-products as functional ingredient to develop a low-glycaemic index bread. *Journal of Cereal Science, 77*, 235–242. https://doi.org/10.1016/j.jcs.2017.08.022

Poutanen, K., Flander, L., & Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology, 26*(7), 693–699. https://doi.org/10.1016/j.fm.2009.07.011

Prückler, M., Lorenz, C., Endo, A., Kraler, M., Dürrschmid, K., HeIiks, K., & Michlmayr, H. (2015). Comparison of homo-and heterofermentative lactic acid bacteria for implementation of fermented wheat bran in bread. *Food Microbiology, 49*, 211–219. https://doi.org/10.1016/j.fm.2015.02.014

Reque, P. M., Pinilla, C. M. B., Tinello, F., Corich, V., Lante, A., Giacomini, A., & Brandelli, A. (2020). Biochemical and functional properties of wheat middlings bioprocessed by lactic acid bacteria. *Journal of Food Biochemistry, 44*(7), e13262.

Rizzello, C. G., Coda, R., Mazzacane, F., Minervini, D., & Gobbetti, M. (2012). Micronized by-products from debranned durum wheat and sourdough fermentation enhanced the nutritional, textural and sensory features of bread. *Food Research International, 46*(1), 304–313. https://doi.org/10.1016/j.foodres.2011.12.024

Rizzello, C. G., Nionelli, L., Coda, R., De Angelis, M., & Gobbetti, M. (2010). Effect of sourdough fermentation on stabilisation, and chemical and nutritional characteristics of wheat germ. *Food Chemistry, 119*(3), 1079–1089. https://doi.org/10.1016/j.foodchem.2009.08.016

Rossi, S., Parrotta, L., Del Duca, S., Dalla Rosa, M., Patrignani, F., Schluter, O., & Lanciotti, R. (2021). Effect of *Yarrowia lipolytica* RO25 cricket-based hydrolysates on sourdough quality parameters. *LWT-Food Science and Technology, 148*, Article 111760. https://doi.org/10.1016/j.lwt.2021.111760

Sabater, C., Ruiz, L., Delgado, S., Ruas-Madiedo, P., & Margolles, A. (2020). Valorization of vegetable food waste and by-products through fermentation processes. *Frontiers in Microbiology, 11*, Article 581997. https://doi.org/10.3389/fmicb.2020.581997

Saerens, S. M., Delvaux, F. R., Verstrepen, K. J., & Thevelein, J. M. (2010). Production and biological function of volatile esters in Saccharomyces cerevisiae. *Microbial Biotechnology, 3*(2), 165–177. https://doi.org/10.1111/j.1751-7915.2009.00106.x

Saura-Calixto, F. (2011). Dietary fiber as a carrier of dietary antioxidants: An essential physiological function. *Journal of Agricultural and Food Chemistry, 59*(1), 43–49. https://doi.org/10.1093/fqsafe/fyx006

Scarnato, L., Serrazanetti, D. I., Aloisi, I., Montanari, C., Del Duca, S., & Lanciotti, R. (2016). Combination of transglutaminase and sourdough on gluten-free flours to improve dough structure. *Amino Acids, 48*, 2453–2465. https://doi.org/10.1007/s00726-016-2258-4

Shewry, P. R. (2009). The HEALTHGRAIN programme opens new opportunities for improving wheat for nutrition and health. *Nutrition Bulletin, 225–231*. https://doi.org/10.1111/j.1467-3010.2009.01747.x

Singh, J., Metrani, R., Shivanagoudra, S. R., Jayaprakasha, G. K., & Patil, B. S. (2019). Review on bile acids: Effects of the gut microbiome, interactions with dietary fiber, and alterations in the bioaccessibility of bioactive compounds. *Journal of Agricultural and Food Chemistry, 67*(33), 9124–9138. https://doi.org/10.1021/acs.jafc.8b07306

Siroli, L., Giordani, B., Rossi, S., Gottardi, D., McMahon, H., Augustyniak, A., & Lanciotti, R. (2022). Antioxidant and Functional Features of Pre-Fermented Ingredients Obtained by the Fermentation of Milling By-Products. *Fermentation, 8*(12), 722. https://doi.org/10.3390/fermentation8120722

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*(1), 49–55. https://doi.org/10.5344/ajev.1977.28.1.49

Sobota, A., Rzedzicki, Z., Zarzycki, P., & Kuzawińska, E. (2015). Application of common wheat bran for the industrial production of high-fibre pasta. *International Journal of Food Science & Technology, 50*(1), 111–119. https://doi.org/10.1111/ijfs.12641

Spaggiari, M., Ricci, A., Calani, L., Bresciani, L., Neviani, E., Dall'Asta, C., & Galaverna, G. (2020). Solid state lactic acid fermentation: A strategy to improve wheat bran functionality. *LWT-Food Science and Technology, 118*, Article 108668. https://doi.org/10.1016/j.lwt.2019.108668

Spaggiari, M., Dall'Asta, C., Galaverna, G., & del Castillo Bilbao, M. D. (2021). Rice bran by-product: From valorization strategies to nutritional perspectives. *Foods, 10*, 85. Doi: 10.3390/foods10010085.

Squeo, G., Silletti, R., Napoletano, G., Greco Miani, M., Difonzo, G., Pasqualone, A., & Caponio, F. (2022). Characterization and effect of refining on the oil extracted from durum wheat by-products. *Foods, 11*(5), 683. https://doi.org/10.3390/foods11050683

Stevenson, L. E. O., Phillips, F., O'sullivan, K., & Walton, J. (2012). Wheat bran: Its composition and benefits to health, a European perspective. *International Journal of Food Sciences and Nutrition, 63*(8), 1001–1013. https://doi.org/10.3109/09637486.2012.687366

Tagliazucchi, D., Martini, S., & Solieri, L. (2019). Bioprospecting for bioactive peptide production by lactic acid bacteria isolated from fermented dairy food. *Fermentation, 5*(4), 96. https://doi.org/10.3390/fermentation5040096

Tan, J., McKenzie, C., Potamitis, M., Thorburn, A. N., Mackay, C. R., & Macia, L. (2014). The role of short-chain fatty acids in health and disease. *Advances in Immunology, 121*, 91–119. https://doi.org/10.1016/B978-0-12-800100-4.00003-9

The Good Scents Company Information System. Providing information for the Flavor, Fragrance, Food and Cosmetic industries. Retrieved from http://www.thegoodscentscompany.com/index.html. Accessed May 25, 2023.

Vermeulen, N., Czerny, M., Gänzle, M. G., Schieberle, P., & Vogel, R. F. (2007). Reduction of (E)-2-nonenal and (E, E)-2, 4-decadienal during sourdough fermentation. *Journal of Cereal Science, 45*(1), 78–87. https://doi.org/10.1016/j.jcs.2006.07.002

Verni, M., Rizzello, C. G., & Coda, R. (2019). Fermentation biotechnology applied to cereal industry by-products: Nutritional and functional insights. *Frontiers in Nutrition, 6*, 42. https://doi.org/10.3389/fnut.2019.00042

Vuksan, V., Sievenpiper, J. L., Jovanovski, E., Jenkins, A. L., Komishon, A., Au-Yeung, F., & Smircic-Duvnjak, L. (2020). Effect of soluble-viscous dietary fibre on coronary heart disease risk score across 3 population health categories: Data from randomized, double-blind, placebo-controlled trials. *Applied Physiology, Nutrition, and Metabolism, 45*(7), 801–804. https://doi.org/10.1139/apnm-2019-0728

Wrigley, C. W., Corke, H., Seetharaman, K., & Faubion, J. (Eds.). (2015). *Encyclopedia of Food Grains*. New York, NY, USA: Academic Press.

Xiong, R. G., Zhou, D. D., Wu, S. X., Huang, S. Y., Saimaiti, A., Yang, Z. J., & Li, H. B. (2022). Health benefits and side effects of short-chain fatty acids. *Foods, 11*(18), 2863. https://doi.org/10.3390/foods11182863

Zhang, J., Liu, M., Zhao, Y., Zhu, Y., Bai, J., Fan, S., & Xiao, X. (2022). Recent developments in fermented cereals on nutritional constituents and potential health benefits. *Foods, 11*(15), 2243. https://doi.org/10.3390/foods11152243

Zhang, M., Chen, X., Hayat, K., Duhoranimana, E., Zhang, X., Xia, S., Yu, J., & Xing, F. (2018). Characterization of odor-active compounds of chicken broth and improved flavor by thermal modulation in electrical stewpots. *Food Research International, 109*, 72–81. https://doi.org/10.1016/J.FOODRES.2018.04.036

Zhao, H. M., Guo, X. N., & Zhu, K. X. (2017). Impact of solid state fermentation on nutritional, physical and flavor properties of wheat bran. *Food Chemistry, 217*, 28–36. https://doi.org/10.1016/j.foodchem.2016.08.062

Zotta, T., Piraino, P., Ricciardi, A., McSweeney, P. L., & Parente, E. (2006). Proteolysis in model sourdough fermentations. *Journal of Agricultural and Food Chemistry, 54*(7), 2567–2574. https://doi.org/10.1021/jf052504s