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Starch-Gluten and Wheat Derivatives as Functional Fortifiers of Sourdough Bread: Nutritional, Antioxidant and Quality Insights

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Abstract

Fortifying sourdough bread with functional ingredients is an effective strategy to enhance nutritional value, bioactive content, and sensory quality. In this study, the novel strain *L. paracasei* SP5 was incorporated into mother sponges together with trahanas (2% and 5% w/w) or delignified wheat bran (2% and 5% w/w) and the effects on sourdough bread functionality were evaluated. Sourdough breads supplemented with trahanas exhibited the highest lactic acid bacteria counts (up to 10.4 log cfu/g) and the strongest acidification (pH 4.25–4.32; TTA 9.1–9.8 mL NaOH), along with elevated lactic (2.50–2.53 g/kg) and acetic acid (2.76–3.11 g/kg) concentrations. These breads also showed enhanced total phenolic content (up to 112.1 mg GAE/100 g) and antioxidant activity (ABTS 221.5 mg TE/100 g; DPPH 5.0 μmol TE/g), as well as phytic acid degradation exceeding 91%, improving mineral bioavailability. Sourdough breads supplemented with wheat bran displayed moderate improvements in these parameters, while control breads had the lowest values. Sourdough breads supplemented with trahanas also demonstrated extended shelf-life. These results indicate that *L. paracasei* SP5 combined with nutrient-rich substrates effectively enhances sourdough bread quality and functionality.

Keywords: *Lacticaseibacillus paracasei* SP5; sourdough bread; trahanas; wheat bran; fortification; nutritional value

1. Introduction

The incorporation of functional ingredients into bread is increasingly recognized as an effective strategy to enhance nutritional value and address dietary deficiencies [1]. In particular, the main particular reasons for bread enrichment are the restoration of nutrients lost during milling, the prevention of deficiencies, the support of energy metabolism and the promotion of overall health. Since bread is a staple food, enrichment ensures essential nutrient intake with minimal dietary changes. Many countries legally mandate and regulate bread fortification, including with bioactive compounds [2,3]. Bread can be supplemented with various ingredients containing vitamins, minerals, dietary fiber, proteins, bioactive compounds and omega-3 fatty acids, resulting in a more nutritionally valuable food product [4,5]. However, fortification can influence bread properties. For instance, high fiber and protein levels may interfere with gluten network formation, leading to denser crumbs [6]. Likewise, proper optimization of formulations and processing, such as adjusting hydration or using enzymes, is essential to balance nutrition with technological and sensory quality.

Although numerous studies have explored the effects of functional ingredients fortification in bread quality, most of them have examined their application in baker's yeast-bread products rather than in sourdough bread. Sourdough fermentation has been established as a natural method that enhances the quality, nutritional value, and shelf-life of bread [7,8]. The incorporation of defined lactic acid bacteria (LAB) as starter cultures allows the control of the fermentation, which leads to increased production of organic acids, improved dough rheology and desirable flavor development [9]. Among LAB, *Lacticaseibacillus paracasei* strains have gained attention, due to their robust fermentation capacity and ability to improve bread functionality by enhancing antioxidant activity and reducing antinutritional factors such as phytic acid [10,11].

Recent studies have shown that the application of free or immobilized *L. paracasei* SP5 in trahanas or delignified wheat bran significantly improves the functional properties of sourdough bread [12]. Specifically, breads prepared with these immobilized cells exhibited increased acidity, higher organic acid concentrations and greater reduction in phytic acid content compared to control breads The improved functionality was attributed to the enhanced metabolic activity of the immobilized *L. paracasei* SP5 within the trahanas or wheat bran matrices. This approach highlighted the potential of using immobilized LAB cells in functional matrices to produce high-quality sourdough breads. However, even though immobilization can enhance the viability of cell in the food fermented matrix, application of free cells are simpler, more flexible, and often more metabolically active in sourdough fermentation. In addition, immobilization requires additional materials (e.g., alginate or gels) and extra preparation steps, making the process more labor-intensive, costly, and less practical for routine bakery-scale production. In the current study, the same substrates previously applied were used; however, no immobilization of LAB was performed**.** This choice was made to simplify the fermentation process and focus on evaluating the direct effects of free LAB cells on bread functionality. By avoiding immobilization, we aimed to observe natural interactions between the bacteria and the dough matrix, providing insights into how *L. paracasei* can enhance antioxidant activity, nutritional value, and flavor under straightforward, practical baking conditions. Likewise, functional ingredients such as trahanas, a traditional fermented wheat product, and delignified wheat bran could serve as additional nutrient sources for LAB and enrich bread with bioactive compounds, including phenolics and dietary fiber.

Likewise, the scope of the current study is to assess the effect of incorporating *L. paracasei* SP5 with trahanas (2% w/w and 5%w/w) or wheat bran (2% w/w and 5% w/w) into sourdough bread production as separate substates by not applying immobilizations. In particular, the fortification impact on physicochemical parameters, organic acid profile, total phenolic content, antioxidant activity, phytic acid degradation, shelf-life, and sensory quality of the produced sourdough breads, will be addressed and discussed. In addition, possible differences between other work applying trahanas and wheat bran as immobilization supports will be discussed.

2. Materials and Methods

2.1. Microorganism and Raw Materials

The potential probiotic strain *L. paracasei* SP5 was cultivated in MRS broth at 37 °C for 48 h. Following incubation, cells were harvested by centrifugation using a Sigma 3K12 centrifuge (Bioblock Scientific, France) at 5000 rpm for 10 min at 25 °C. All culture media were sterilized prior to use by autoclaving at 120 °C for 15 min under a pressure of 1–1.5 atm.

Commercial white wheat flour (Hellenic Biscuit Co. S.A., Greece) was employed for bread production, with the following composition (% w/w): protein 11.0, carbohydrates 72.0, fat 1.5, fiber 2.2, and moisture 12.0. Baker’s yeast was a commercial pressed product (S.I. Lesaffre, France) containing 70% (w/w) moisture.

Trahanas and delignified wheat bran were used as supplements in the present study during the sourdough preparation. Likewise, trahanas was prepared by mixing 70% hard wheat flour with sour sheep’s milk, boiling, soaking in fresh sour milk, drying/maturing for 4 days at 30 °C, and cutting into ~1 cm^3 cubes [13]. Wheat bran consisted of approximately 50% dietary fibre, 20% protein, 7% ash, and 4% lipids. Initially, it was delignified by boiling with NaOH solution and sterilised by autoclaving at 120 ◦C, 1–1.5 atm for 15 min [14].

2.2. Freeze-Drying

Freeze-drying of *L. paracasei* SP5, trahanas and wheat bran, was conducted by freezing at −44 °C (5 °C/min) and drying for 48 h (at 5–15 mbar and −45 °C) with a FreeZone 4.5 Freeze-Drying System (Labconco, Kansas City, MO, USA) [12].

2.3. Sourdough Bread Making

Sourdough bread was produced by mixing all ingredients and kneading manually. The dough was moulded in 1.5 L baking pans. Initially, 5 mother sponges were prepared by mixing for 15 min 300 g wheat flour and 160 ml tap water with

(i) 1% w/w (on flour basis) of freeze-dried *L. paracasei* SP5 and 2% freeze dried trahanas (LPT2)
(ii) 1% w/w (on flour basis) of freeze-dried *L. paracasei* SP5 and 5% freeze dried trahanas (LPT5)
(iii) 1% w/w (on flour basis) of freeze-dried *L. paracasei* SP5 and 2% freeze dried delignified wheat bran (LPWB2)
(iv) 1% w/w (on flour basis) of freeze-dried *L. paracasei* SP5 and 5% freeze dried delignified wheat bran (LPWB5))

Then, 5 sourdough breads were produced containing 30% w/w (on flour basis) of the aforementioned mother sponges, respectively. In particular, 5 types of sourdough breads were produced containing sourdough: LPT2 (SB1), LPT5 (SB2), LPWB2 (SB3) and LPWB5 (SB4). In addition, a traditional sourdough (wild microflora) provided by a local bakery was also used for the production of the Control sourdough bread (SB5) in the same amount with the other sourdoughs (30% w/w). The formulation of all the sourdough breads was the same: 150 g of each sourdough, 500 g wheat flour, approximately 270 ml tap water, 4 g salt and 1% w/w (on flour basis) pressed baker's yeast. All doughs were fermented at 30 ^{o}C for 2 h, proofed at 40 ^{o}C for 60 min, and baked at 230 ^{o}C for ~40 min. All trials were carried out in triplicate.

2.4. Analytical Methods

2.4.1. Microbial Cell Counts and Monitoring of Bread Spoilage

The determination of viable microbial populations, expressed as colony-forming units per gram (cfu/g), was performed by aseptically suspending 1 g of each sample in 9 mL of phosphate buffer (0.25 M KH_2PO_4, prepared by diluting 1.25 mL of the stock solution per liter of distilled water). Serial decimal dilutions were prepared and plated on de Man, Rogosa, and Sharpe (MRS) agar (Fluka, Buchs, Switzerland), followed by incubation at 37 °C for 48–72 h to enumerate lactic acid bacteria. In the case of sourdough samples, viable cell counts of LAB and yeasts were determined after homogenizing 20 g of dough in 200 mL of phosphate buffer. Yeast enumeration was conducted on malt extract agar (Fluka, Buchs, Switzerland) after incubation at 30 °C for 48 h.

Bread loaves were stored at ambient temperature in sterile polyethylene bags and systematically examined for spoilage development, as previously described by [17]. The appearance of visible fungal colonies on the bread surface was recorded as the time of mold spoilage onset. Rope spoilage assessment was based on characteristic macroscopic and sensory indicators, including the development of a sweet, fruity or overripe melon-like odor, the occurrence of crumb discoloration, and the formation of sticky, thread-like filaments within the crumb structure. These parameters collectively provided an integrated evaluation of microbial stability and spoilage progression during bread storage. All determinations were carried out in triplicate.

2.4.2. Organic Acids

The quantification of organic acids (lactic, acetic, formic, propionic, *n*-valeric, and caproic acids) present in the sourdough breads was performed using high-performance liquid chromatography (HPLC), following the method previously described by [12]. In brief, 10 g of each bread sample were homogenized with 90 mL of sterile distilled water using a Seward Stomacher 400 blender (London, UK). The resulting homogenates were centrifuged, and the supernatants were collected for chromatographic analysis.

Analyses were conducted on a Shimadzu HPLC system equipped with a Shim-pack IC-A1 analytical column, an LC-10AD pump, a CTO-10A column oven maintained at 40 °C, and a CDD-6A conductivity detector. The mobile phase consisted of phthalic acid (2.5 mM) and tris(hydroxymethyl)aminomethane (2.4 mM) adjusted to pH 4.0, delivered at a constant flow rate of 1.2 mL/min. This analytical configuration allowed accurate separation and quantification of the principal organic acids contributing to the biochemical profile of the sourdough breads.

Determination of the organic acid concentrations was carried by means of standard curves.

2.4.3. pH and Total Titratable Acidity (TTA)

The pH of sourdough bread samples was measured using a Sentron Argus pH meter (Sentron Europe B.V., Roden, Netherlands). Total titratable acidity (TTA) was determined as the volume of 0.1 M NaOH (mL) required to neutralize 10 g of breadcrumb. For this purpose, 10 g of breadcrumb were homogenized with 90 mL of deionized water, and the supernatant was titrated with 0.1 M NaOH until a pH endpoint of 8.5 was reached [12]. This analysis provided a quantitative measure of the overall acidity of the sourdough, reflecting the metabolic activity of lactic acid bacteria and other fermentative microorganisms.

2.4.4. Specific Loaf Volume

The specific loaf volume (mL/g) of the sourdough breads was assessed using the rapeseed displacement method [12].

2.4.5. Total Phenolic Content (TPC)

Following baking, the bread loaves were allowed to cool at room temperature for 3 h. Crumb samples were then freeze-dried for 48 h using a FreeZone 4.5 lyophilizer (Labconco, Kansas City, MO, USA). For extraction, 1 g of the freeze-dried crumb was suspended in 20 mL of phosphate-buffered saline (PBS, pH 7.4) and incubated under continuous shaking at 37 °C for 1 h. The residue was subjected to a second extraction with an additional 20 mL of PBS. The resulting supernatants were pooled and stored at −20 °C until subsequent determination of total phenolic content (TPC) and antioxidant capacity (AC).

Total phenolic content (TPC) was quantified using the Folin–Ciocalteu reagent method, with slight modifications to the procedure described previously [15]. In brief, 200 µL of each bread crumb extract was mixed with 800 µL of Folin–Ciocalteu reagent and allowed to react in the dark for 2 min. Subsequently, 2 mL of 7.5% (v/v) sodium carbonate solution were added, and the reaction volume was adjusted to 10 mL with distilled water. The mixture was then incubated in the dark at room temperature for 60 min. Absorbance was measured at 765 nm using a spectrophotometer. Standard gallic acid (GA) solutions and blanks were prepared concurrently. TPC values were expressed as milligrams of gallic acid equivalents per 100 g of dried sample (mg GAE/100 g).

2.4.6. Antioxidant Capacity (AC)

The antioxidant capacity of the sourdough bread extracts was evaluated using both the ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation assay, expressed as Trolox Equivalent Antioxidant Capacity (TEAC), and the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. For the ABTS assay, the $ABTS^{\bullet +}$ stock solution was prepared by combining equal volumes of 7.4 mM ABTS and 2.6 mM potassium persulfate in water, followed by incubation at room temperature for 12 h to allow complete radical formation. The solution was then diluted with distilled water to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Subsequently, 100 µL of bread extract was mixed with 3.9 mL of the diluted $ABTS^{\bullet +}$ solution, incubated for 4 min, and the absorbance was measured at 734 nm against a blank.

For the DPPH assay, the radical scavenging activity was determined by reacting the extracts with a DPPH solution, and the decrease in absorbance at 517 nm was monitored, reflecting the capacity of the extract to quench free radicals. In both assays, standard curves were prepared using Trolox, and the results were expressed as milligrams of Trolox equivalents per 100 g of dried sample (mg TE/100 g), providing a quantitative measure of the overall antioxidant potential of the sourdough breads [15].

Regarding the DPPH assay, the extracts were mixed with DPPH solution and left to react in the dark for 60 min. The absorbance of the samples was then recorded at 515 nm. In both assays, antioxidant capacity was quantified using a Trolox calibration curve, and results were expressed as Trolox equivalents (mol TE/g dry weight), providing a standardized measure of the free radical–scavenging potential of the sourdough bread extracts.

2.4.7. Phytic Acid

Phytic acid (phytate; myo-inositol-1,2,3,4,5,6-hexakisphosphate) content in the sourdough bread samples was quantified by measuring the phosphorus released through enzymatic hydrolysis, using the Megazyme K-PHYT assay kit (Megazyme, Bray, Ireland) in accordance with the manufacturer's instructions.

2.4.8. Statistical Analysis

Analysis of Variance (ANOVA) followed by Duncan's post hoc multiple range test was applied to extract the specific differences between the various treatments, i.e., the effects of the different sourdoughs on the physicochemical and sensory characteristics of the produced breads. The analysis was performed using the SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA) software at an alpha level of 5%.

3. Results and Discussion

3.1. Acidity Levels

The incorporation of *L. paracasei* SP5 with trahanas or delignified wheat bran significantly enhanced the metabolic activity of the sourdough ecosystem (Table 1). The higher LAB levels in SB1 and SB2—particularly in trahanas sourdoughs—confirm that SP5 rapidly adapts to cereal-based substrates rich in carbohydrates, peptides, and organic acids [13,15]. Trahanas is a fermented dairy–grain matrix containing lactose derivatives, organic acids, and amino acids; likewise, it offers a favor fermentability environment, explaining the higher LAB growth. These findings are consistent with previous research demonstrating that substrate composition can directly modulate LAB proliferation and metabolic performance, particularly when peptides and fermentable sugars coexist [16,17]. Although wheat bran contains mostly structurally complex dietary fibers that are difficult for microbes to use quickly, it still supported moderate LAB growth. This can be attributed to the gradual liberation of fermentable carbohydrates from its complex fiber matrix, along with the release of bound phenolics during fermentation [18]. These components not only serve as slow-acting energy sources but may also enhance LAB metabolism through their bioactive functions.

Table 1. Microbial counts (Log cfu/g) of Lactic acid bacteria (LAB).and yeasts in sourdoughs containing *L.paracasei* SP5 with trahanas.or wheat bran.

Sourdough	LAB	Yeasts
	Log cfu/g	
SB1	9.2 ± 0.1^{c}	8.2 ± 0.1^{a}
SB2	10.4 ± 0.1^{a}	8.3 ± 0.2^{a}
SB3	8.7 ± 0.1^{c}	7.8 ± 0.2^{b}
SB4	9.9 ± 0.2^{b}	8.1 ± 0.1^{a}
SB5	8.5 ± 0.1^{c}	7.7 ± 0.2^{c}

LAB: Lactic acid bacteria. cfu: Colony forming units. Different superscript letters in a.column, indicate statistically significant differences ($p<0.05$).

3.2. Physicochemical Characteristics of Breads

Breads made with trahanas (SB1, SB2) exhibited significantly lower pH values (4.25–4.32) and higher total titratable acidity (9.1–9.8 mL NaOH), indicating vigorous fermentation, whereas bran-enriched breads showed intermediate acidity, and the control SB5 showed the weakest acidification (Table 2).

This strong acidification capacity is consistent with the known heterofermentative metabolism of *L. paracasei*, which yields lactic and acetic acids through both phosphoketolase and citrate metabolism pathways [7,19]. Indeed, the lactic–acetic acid balance detected in SB1 and SB2 suggests enhanced flux through these pathways under nutrient-rich conditions provided by trahanas.

Acetic acid levels were significantly higher in SB2, a desirable outcome because acetic acid improves bread aroma, enhances shelf-life, and inhibits fungal growth [20]. Research also shows that more complex matrices containing peptides and organic acids stimulate LAB metabolite production, leading to improved bread stability and flavor development [21].

A major finding is that *L. paracasei* SP5 cells outperformed or matched the acidification efficiency of previously immobilized *L. paracasei* SP5 systems [12]. Immobilization typically enhances metabolic stability by protecting cells from stress, but here, free freeze-dried cells paired with trahanas provided superior lactic and acetic acid yields.

This finding challenges prevailing assumptions that immobilization is required for maximal acid production and agrees with studies showing that certain LAB strains retain high metabolic activity post–freeze-drying when rehydrated in nutrient-rich environments [22].

Table 2. Physicochemical characteristics of breads made with sourdoughs prepared with freeze-dried, free and immobilized, *L. paracasei* SP5 and with the control sourdough.

Bread sample	**pH**	**TTA (ml 0.1 M NaOH)**	**SLV (ml/g)**	**Organic acids (g/Kg bread)**				
				Lactic	**Acetic**	**Formic**	**n-Valeric**	**Caproic**
SB1	4.32 ± 0.03^{c}	9.12 ± 0.09^{b}	2.50 ± 0.05^{a}	2.85 ± 0.07^{b}	1.08 ± 0.02^{b}	0.08 ± 0.01^{b}	0.06 ± 0.01^{b}	0.04 ± 0.01^{b}
SB2	4.25 ± 0.05^{c}	9.79 ± 0.10^{a}	2.53 ± 0.06^{a}	3.11 ± 0.06^{a}	1.15 ± 0.02^{a}	0.09 ± 0.01^{a}	0.08 ± 0.01^{a}	0.06 ± 0.01^{a}
SB3	4.48 ± 0.05^{b}	8.91 ± 0.11^{c}	2.52 ± 0.05^{a}	2.63 ± 0.05^{c}	0.97 ± 0.02^{c}	0.04 ± 0.03^{b}	0.04 ± 0.01^{b}	0.03 ± 0.01^{b}
SB4	4.40 ± 0.05^{b}	9.07 ± 0.09^{b}	2.50 ± 0.04^{a}	2.76 ± 0.05^{c}	0.97 ± 0.02^{c}	0.06 ± 0.03^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}
SB5	4.70 ± 0.05^{a}	6.80 ± 0.08^{d}	2.51 ± 0.05^{a}	2.45 ± 0.06^{d}	0.82 ± 0.02^{d}	0.03 ± 0.02^{c}	tr	tr

TTA: total titratable acidity; SLV: specific loaf volume; tr: traces (<0.01 g/Kg); Different superscript letters in a column indicate statistically significant differences (ANOVA, Duncan's multiple range test, $p<0.05$.

3.3. Shelf-Life of Breads

Shelf-life measurements revealed that SB2 (13 days mould resistance; 12 days rope resistance) and SB1 (11.5 and 10.5 days) exhibited the strongest preservation, followed by SB4, SB3, and finally SB5, which spoiled after only 7–7.5 days (Table 3).

The extended shelf-life of SB1 and SB2 is attributed to high LAB counts and the production of antifungal metabolites such as organic acids, phenyl-lactic acid, hydroxy-fatty acids, and bacteriocins [23]. Additionally, LAB fermentation lowers water activity and redox potential, creating conditions unfavorable for mould development [24].

Recent studies have shown that sourdough metabolites, especially acetic acid and phenolic derivatives, act synergistically to inhibit spoilage organisms through membrane disruption and intracellular pH collapse [25,26]. The performance of free SP5 cells was comparable to immobilized systems reported previously, demonstrating that immobilization is not necessary to achieve strong biopreservative effects when functional substrates like trahanas are included.

Table 3. Appearance of rope and mould spoilage.

Bread sample	Mould spoilage	Rope spoilage
	days	
SB1	11.5 ± 0.5^b	10.5 ± 0.5^b
SB2	13.0 ± 0.5^a	12.0 ± 0.5^a
SB3	10.5 ± 0.5^b	9.5 ± 0.5^b
SB4	11.5 ± 0.5^a	10.5 ± 0.5^b
SB5	7.5 ± 0.5^c	7.0 ± 0.5^c

Different superscript letters in a column indicate statistically significant differences (ANOVA, Duncan's multiple range test, $p<0.05$.

3.4. TPC and AA

Fortified breads, especially SB2 and SB1, showed significantly elevated total phenolic content (TPC) and antioxidant activity (Table 4). These findings align with evidence showing that LAB fermentation enhances phenolic bioaccessibility by releasing bound phenolics via esterases and glycosidases [9]. Trahanas matrices amplified these effects by supplying additional microbial-accessible nutrients that stimulate enzyme expression, resulting in superior antioxidant profiles relative to bran breads. Bran breads still outperformed the control due to their intrinsic phenolic richness but were limited by fiber barriers that reduce extractability. Numerous studies confirm that LAB strains capable of phenolic transformation can significantly increase antioxidant activity in sourdough breads by releasing ferulic acid, p-coumaric acid, and other bound phenolics from cereal matrices[27,28]. The high ABTS and DPPH values of SB2 demonstrate considerable radical-scavenging capacity, supporting the role of SP5 in enhancing the bread's functional properties.

Table 4. Total phenolic content (TPC) and antioxidant activity (AA) (on dry weight basis) of the produced sourdough breads.

Bread Sample	TPC (mg GAE/100 g)	ABTS (mg TE/100 g)	DPPH (μmol TE/g)
B1	91.7 ± 2.4^c	199.3 ± 3.5^b	4.4 ± 0.1^b
SB2	112.1 ± 1.9^a	221.5 ± 3.9^a	5.0 ± 0.1^a
SB3	88.4 ± 3.2^c	181.2 ± 3.0^c	3.9 ± 0.2^c
SB4	99.4 ± 4.1^b	201.1 ± 3.1^b	4.4 ± 0.1^b
SB5	64.7 ± 2.9^d	174.6 ± 1.9^d	3.3 ± 0.1^d

Different superscript letters in a column indicate statistically significant differences (ANOVA, Duncan's multiple range test, $p<0.05$.

3.5. Phytic Acid Content

Phytic acid reduction exceeded 90% in SB1 and SB2, compared with 75.6% in the control (Table 5). This reduction results from two synergistic mechanisms: (i) LAB-produced phytases hydrolyze phytate and (ii) acidic sourdough conditions activate endogenous cereal phytases, optimally active at pH 4.0–5.5 [29].

SB2 showed the strongest reduction due to enhanced fermentation acidity combined with trahanas-derived nutrients stimulating SP5 phytase expression, consistent with findings in other LAB-fermented cereals [30].

Comparison with immobilized SP5 systems revealed that free SP5 achieved equal or superior phytate degradation—an important industrial consideration since immobilization increases production cost and complexity without additional nutritional benefit [12].

Recent studies further support that free LAB combined with nutrient-rich substrates can outperform immobilized counterparts when fermentation conditions favor enzyme expression [29,31].

Table 5. Phytic acid content.

Bread sample	Initial Phytic Acid (mg/g dw)	Phytic Acid after Baking (mg/g dw)	Reduction (%)
SB1	4.5 ± 0.05	0.5 ± 0.01^{c}	88.9^{b}
SB2	4.6 ± 0.05	0.4 ± 0.01^{c}	91.3^{a}
SB3	4.4 ± 0.05	0.7 ± 0.01^{b}	84.1^{c}
SB4	4.5 ± 0.05	0.7 ± 0.01^{b}	84.4^{c}
SB5	4.5 ± 0.05	1.1 ± 0.01^{a}	75.6^{d}

Different superscript letters in a column indicate statistically significant differences (ANOVA, Duncan's multiple range test, $p<0.05$.

3.6. Challenges and Research Gaps

Even though, the fortification of sourdough breads with *L. paracasei* SP5 combined with trahanas or wheat bran demonstrates promising improvements in functional, nutritional and sensory properties, yet several technological challenges remain. A central limitation concerns the stability and viability of free LAB cells during storage, processing, and baking. Immobilized LAB systems typically confer adequate protection against thermal, oxidative, and osmotic stress, resulting in enhanced long-term survival compared with free cells [32]. In contrast, the performance of free cultures depends strongly on matrix composition, rehydration kinetics, and environmental stressors encountered during dough preparation and baking.

Another challenge lies in the reproducibility and predictability of fermentation kinetics. Immobilized cells generally provide more uniform acidification profiles, whereas free-cell systems may exhibit variability in metabolic distribution within the dough matrix. This variability can influence acidification rate, organic acid ratios, and metabolite formation—factors that become critical during industrial-scale production where batch homogeneity is essential. A further research gap concerns the interaction between free *L. paracasei* SP5 cells and complex substrates such as trahanas or wheat bran. Unlike immobilized systems where microbial localization is fixed, free cells interact heterogeneously with dough components, potentially influencing the release of volatile compounds, phenolics, and other bioactives. Although sensory outcomes were favorable in this study, the underlying biochemical mechanisms remain insufficiently characterized.

Finally, although free-cell fortification strategies reduce processing complexity and cost compared with immobilization, their performance under industrial scaling—including large-scale mixing, prolonged proofing, and mechanical stress—has not been fully evaluated. Comparative studies examining microbial stability, nutrient bioavailability, and shelf-life under real-world bakery conditions are needed to determine the robustness of *L. paracasei* SP5 fortification relative to immobilized systems.

4. Conclusions

Overall, the results indicate that free *L. paracasei* SP5 cells in combination with trahanas or wheat bran can significantly improve the technological, nutritional and sensory attributes of sourdough bread. The finding that *L. paracasei* SP5 performs comparably to immobilized systems is technologically relevant, as immobilization adds cost, complexity and additional processing steps. Even though, immobilization is known to increase cell protection and viability during storage, the present results show that free cells can achieve similar outcomes when supported by suitable substrates. Future work should include comprehensive metabolomic profiling, rheological analysis and industrial-scale validation to determine the long-term potential of free *L. paracasei* SP5 fortification strategies in commercial sourdough bread production. Overall, the results indicate that fermentation with *L. paracasei* SP5 combined with functional substrates like trahanas or wheat bran (particularly at 2–5% w/w addition levels) significantly enhances the quality, nutritional value and shelf-life of sourdough bread. This approach represents a promising strategy for developing functional breads with improved consumer acceptance and health-promoting properties.

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