



Original research

Effect of fermented sprouted amaranth on quality characteristics of synbiotic wheat bread

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A B S T R A C T

The present study was conducted to investigate the effect of using controlled fermentation of sprouted amaranth sourdough (SASD) on the techno-functional properties of synbiotic wheat bread. Based on the results obtained, the total titratable acidity of the produced sourdough stabilized after five successive back-slopping cycles, and the predominant lactic acid bacteria (LAB) were isolated from it. The antifungal activity of one of the predominant LAB isolates of this sourdough against *Aspergillus flavus* was significantly ($p < 0.05$) higher than that of the other isolates; therefore, it was selected as the starter culture for controlled sourdough fermentation. Sequencing analysis of the PCR products identified the selected LAB isolate as *Lactocaseibacillus casei*. Texture analysis results showed that the sample containing SASD exhibited the lowest crumb hardness and the highest specific volume compared with wheat breads containing sprouted amaranth, amaranth flour and the control sample. The aforementioned sample also demonstrated the highest antioxidant activity (72.90%) and the lowest surface fungal growth (6.92%). In addition, the survival rate of the probiotic *Bacillus coagulans* after baking was significantly higher in the bread containing SASD than in the control sample. Based on these findings, controlled fermentation of SASD can be applied in the production of functional wheat bread.

Keywords: Sprouted amaranth; Sourdough; Synbiotic bread; Antifungal effect.

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1. Introduction

Probiotics are live, metabolically active microorganisms that if you take them in sufficient amounts (10^6 – 10^8 CFU/g) can really start doing beneficial health effects on the host. These benefits include regulation of the intestinal microbial flora, inhibition of pathogenic microorganisms, and modulation of immune system responses (Sadeghi et al., 2022). Although dairy products are considered the most important probiotic products in the food industry, their consumption is limited due to allergenic proteins, the need for cold storage, lactose intolerance in some individuals, the presence of animal cholesterol, and the increasing demand for plant-based products. Therefore, non-dairy alternatives with better probiotic delivery potential are increasingly being developed. Cereal-based probiotic products can serve as suitable substitutes for dairy products. Among cereal products, bread has special importance.

Bread, as a staple food for most people worldwide, is one of the oldest food products and, besides its low cost, is an excellent source of carbohydrates and micronutrients. Moreover, the production of functional breads is easily achievable due to their enrichment capability with vitamins, minerals, phenolic compounds, microorganisms and enzymes (Sadeghi et al., 2024a).

Several approaches exist for incorporating probiotics into bread, including direct addition. However, this method may cause undesirable flavors and sensory defects, limiting its application. Furthermore, high baking temperatures destroy most probiotic microorganisms (Tolve et al., 2022). Microencapsulation is another strategy that protects probiotics and may improve physicochemical and rheological properties of bread, but it requires specific coating materials and increases production costs. Similarly, edible coatings may dry during storage or reduce probiotic viability. Considering these limitations, the use of thermotolerant probiotic bacteria such as *Bacillus coagulans* has emerged as one of the most effective

Abbreviations: LAB: lactic acid bacteria, MRS: de Man, Rogosa and Sharpe, PDA: potato dextrose agar, SASD: sprouted amaranth sourdough, TTA: total titratable acidity.

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methods for producing probiotic bread (Mani-López et al., 2023). Synbiotic formulations as combined application of probiotic microorganisms and prebiotic ingredients have received considerable attentions in developing bakery products with health-promoting potentials. Production of synbiotic bread containing probiotics and prebiotics (such as amaranth) may also enhance probiotic survival during processing, storage and gastrointestinal transit while improving bread quality indices (Beikzadeh et al., 2025).

Amaranth, belonging to the *Amaranthaceae* family, is a pseudo-cereal with approximately 60 species. It is rich in lipids, proteins, carbohydrates and dietary fibers. It contains 9.5–16.8% protein, high levels of essential amino acids such as methionine and lysine, minerals and vitamins, especially magnesium and calcium. Amaranth also contains bioactive compounds including lectins, antimicrobial peptides, protease inhibitors and antioxidants that inhibit free radicals and oxidative reactions. Additional health benefits include reduction of blood cholesterol and glucose levels, alleviation of hypertension and anemia, and immune system stimulation (Caselato-Sousa & Amaya-Farfán, 2012). Generally, sprouting and fermentation can also improve the functional properties, nutritional value and prebiotic potential of pseudocereals (Pahlavani et al., 2024; Zarali et al., 2024).

Several studies have been conducted in Iran on probiotic and synbiotic breads. For instance, Yousefi et al. (2017) microencapsulated *Lactobacillus plantarum* A7 using sodium alginate and corn starch to produce probiotic bread. Ghasemi et al. (2022) used encapsulated *Lactobacillus acidophilus* and *L. plantarum* in gluten-free bread, improving both probiotic survival and moisture retention. Ganjoori et al. (2012) produced probiotic bread using thermotolerant *B. coagulans* without encapsulation, demonstrating high resistance to heat, low pH, bile salts and antibiotics. Farajinejad et al. (2023) used *B. coagulans* IBRC-M 10807 in the production of bread containing exopolysaccharides. The added fructo-oligosaccharides had a favorable effect on increasing the biomass of *B. coagulans* and, together with this probiotic bacterium, reduced starch retrogradation and produced sourdough bread with improved anti-staling properties. Majzoobi et al. (2019) used *B. coagulans* GanedenBC30 in the production of synbiotic bread containing inulin. The bread, supplemented with 10^9 spores/g, was baked using two methods: frozen par-baked dough and non-frozen dough. The bacterial count in the non-frozen bread was approximately 10^7 CFU/g, while the results of counting *B. coagulans* in frozen par-baked bread were slightly higher. Moreover, storing the frozen par-baked breads for 3 days at 20 °C had no significant effect on the bacterial count.

Recently, several reports have been presented worldwide regarding probiotic bread production. For example, Almada-Érix et al. (2022) reported that in bread made from wheat flour and white flour containing *B. coagulans* GBI-30 6086 spores, the baking process reduced the spore count from 7 logarithmic cycles per gram to approximately 5.5 logarithmic cycles. The greatest reduction in spores occurred in the crust (about two 2 cycles), while the reduction in the crumb was about 1.5 logarithmic cycles. Additionally, the activity of *B. coagulans* had no effect on the pH, texture, color, water activity or moisture of the produced bread. The physicochemical parameters of the bread were evaluated for up to 10 days after baking, and this bacterium was introduced as a suitable candidate for probiotic bread production. De Bellis et al. (2015), in a study aimed at evaluating the safety and thermal tolerance of spore-forming bacteria in bread, found that among 54 cytotoxic spore-forming bacteria isolated from bread -mainly *Bacillus* species- these were

classified into three groups based on thermal resistance. The first group, such as *Bacillus amyloliquefaciens*, exhibited lower thermal tolerance to the baking process and showed more than a 5-log reduction. The second group, including *Bacillus mojavensis* and *Bacillus subtilis*, showed a 2–5 log reduction in spore numbers. Finally, *Bacillus cereus* spores, with less than a 2-log reduction, demonstrated the highest thermal tolerance among the cytotoxic bacteria under bread processing conditions. Jao et al. (2011) used *B. coagulans* Ganeden BC30 in some cereal products such as toast bread. The pH, acidity and viability of this bacterium were evaluated at 4 °C and 25 °C during 15 days. During this period, the pH of the bread increased, its acidity decreased, and the viability of the bacterium decreased by approximately one logarithmic cycle during storage.

Other studies confirmed high thermal resistance and gastrointestinal survival of *B. coagulans* compared with lactic acid bacteria (LAB). Majeed et al. (2021) conducted a study aimed at comparing the viability of *Lactobacillus* species and *B. coagulans* MTCC 585 under various thermal processes such as Chapatti bread baking. The results showed that the spore-forming bacterium exhibited higher thermal resistance and remained up to 92% viable during baking. Furthermore, its viability was maintained at 73% under microwave baking conditions at 260 °C for 300 seconds. According to Calasso et al. (2023), probiotic species including *Bacillus*, *Bifidobacterium* and *Lactobacillus* have been used in various food products. The resistance of selected probiotic species under simulated gastrointestinal conditions was examined, and the viability of *B. coagulans* in the produced products was reported to be up to 80%. Among the produced food products, bread containing *B. coagulans* showed the lowest reduction in bacterial count (one logarithmic cycle) compared with cheese and orange juice.

Despite these advances, no report has yet investigated the application of sprouted amaranth sourdough (SASD) in synbiotic wheat bread. Therefore, the objective of this study was to evaluate the techno-functional properties of synbiotic wheat bread produced using controlled fermentation of SASD.

2. Materials and Methods

2.1. Preparation of raw materials

Amaranth seeds were sprouted under controlled conditions according to the method described by Elkhaila and Bernhardt (2010). The sprouted amaranth was subsequently milled into flour. The physicochemical properties of the flour, including moisture, fat, protein, ash and carbohydrate contents, were determined according to standard methods (AACC, 2010). White wheat flour and baker's yeast were purchased from a local market. Microbial culture media and chemical reagents used in the present study were also supplied from verified commercial brands with analytical grade.

2.2. Spontaneous sourdough fermentation

For spontaneous fermentation, a dough yield of 200 (dough weight/flour weight $\times$ 100) was used. The prepared dough was incubated at 37 °C for 24 h. Through successive back-slopping cycles, predominant LAB were isolated using surface plating on selective media. To measure pH and total titratable acidity (TTA), 10 g of sourdough was mixed with 90 mL of distilled water and

titrated with 0.1 N NaOH to a final pH of 8.5. TTA was expressed as lactic acid (Sadeghi et al., 2019).

2.3. Isolation of predominant lactic acid bacteria

Ten grams of sourdough were homogenized with 90 mL sterile Ringer's solution. Serially ten-fold dilutions were prepared and plated on de Man, Rogosa and Sharpe (MRS) media. Plates were incubated at 37 °C for 24 h. The isolates were screened for antifungal activity using the overlay method (Magnusson & Schnürer, 2001). Hemolytic activity of the selected isolate was evaluated on blood agar containing 5% defibrinated sheep blood after 48 h incubation at 37 °C.

2.4. Screening of the LAB isolates

After two activation steps, isolates were inoculated onto MRS agar and incubated at 37 °C for 72 h. Subsequently, 1 mL of *Aspergillus flavus* spore suspension (10^6 spores/mL) was mixed with 9 mL of potato dextrose agar (PDA) medium and poured over the plates. After solidification, plates were incubated at 28 °C and inhibition zones were measured relative to the control.

2.5. Molecular identification of the selected isolate

The isolate with the greatest antifungal activity was selected for further studies. Genomic DNA was extracted and the target sequence was amplified using universal primers F44 and R1543. PCR products were confirmed by electrophoresis, and then sequenced for identification (Abnous et al., 2009).

2.6. Controlled fermentation of sprouted amaranth

The selected isolate was cultured in MRS broth at 37 °C for 24 h. Cells were harvested by centrifugation (Sigma, Germany; 10,000 rpm, 4 °C, 10 min), washed with sterile phosphate buffer (pH 7), and re-suspended to obtain approximately 10^8 CFU/g. The suspension was added to sprouted amaranth dough (dough yield 200) and fermented at 37 °C for 24 h (Montemurro et al., 2019).

2.7. Evaluation of synbiotic wheat bread characteristics

2.7.1. Bread processing

The control bread was prepared using wheat flour, 2% baker's yeast and water, followed by fermentation (3 h at 37 °C) and baking at 200 °C for 20 min. For experimental samples, 10% (w/w) controlled fermented SASD was incorporated into the formulation. Additional formulations included probiotic bread containing 10^8 CFU/g of *B. coagulans*, and synbiotic bread containing both fermented SASD and the probiotic strain. All samples were processed under identical conditions.

2.7.2. Probiotic survival after baking

Serially ten-fold dilutions were prepared from each manufactured sample including synbiotic (FSA+PRO) formulation along with positive (C+PRO) and negative (C) control breads after

baking and plated on nutrient agar medium to determine viable cell counts after 24 h incubation at 37 °C. FSA-added sample was also cultured with the same procedure to investigate survivability of the LAB starter culture after baking.

2.7.3. Crumb hardness and specific volume

Crumb hardness was measured using a texture analyzer (Micro Systems, UK) two hours after baking according to Katina et al. (2006). The specific volume of the samples was also determined by means of the rapeseed displacement method.

2.7.4. Surface mold growth

Three microliters of *A. flavus* spore suspension (10^6 spores/mL) were inoculated onto sterile filter discs placed on bread surfaces and incubated at 25 °C for one week. Surface fungal growth was assessed relative to the control (Gerez et al., 2009).

2.7.5. Antioxidant activity

Each bread sample (5 g) was mixed with 80% methanol (50 mL) and then centrifuged at 4600 g for 20 min. The supernatant was used for analysis. Antioxidant activity was determined using the DPPH radical scavenging assay by mixing 2 mL of extract with 2 mL of 0.1 mM ethanolic DPPH solution. Absorbance was measured at 517 nm and inhibition percentage was calculated (Yu et al., 2003).

2.7.6. Overall acceptability

Sensory evaluation was performed two hours after baking. Crust color, flavor, chewiness and aroma were assessed by 10 trained panelists (men and women with 25-50 years old) using a five-point hedonic scale. Accordingly, manufactured samples were categorized using 1-5 scores as the lowest and the highest overall acceptability, respectively (Katina et al., 2006). The obtained data were also presented as overall acceptability (mean values of the studied sensory characteristics) in Table 1.

2.8. Statistical analysis

A completely randomized design was used for the experiment. Data were subjected to one-way analysis of variance (ANOVA), and mean separation was carried out using the least significant difference (LSD) test with a significance level of $p < 0.05$. All analyses were conducted in triplicate using SPSS software, and graphs were prepared using Microsoft Excel 2016.

3. Results and Discussion

3.1. Changes in pH and total titratable acidity during back-slopping processes

Amaranth flour used in this research had 12.4% moisture, 15.7% protein, 3.3% ash, 7.2% fat and 61.4% total carbohydrates. The wheat flour used with 68% extraction rate, also contained 11.2% protein, 0.4% ash and 14.2% moisture. As shown in Fig. 1, after four successive back-slopping cycles, the TTA of the SASD stabilized at approximately pH 3.95, and no significant difference ($p > 0.05$) was

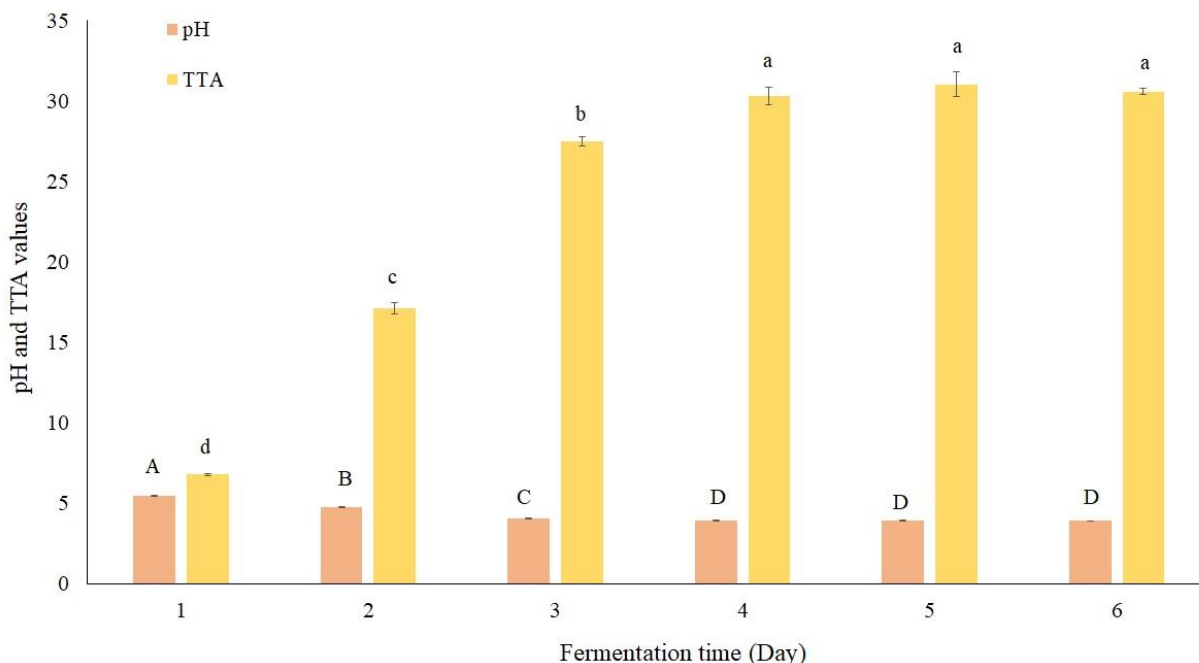


Fig. 1. Changes in pH and total titratable acidity (TTA) of sprouted amaranth sourdough during six days of the successive back-slopping process. Different lowercase and uppercase letters indicate statistically significant differences ($p < 0.05$) among TTA and pH values, respectively. This chart was also constructed using $SD \pm$ mean values.

observed among the TTA values from the fourth to the sixth day of back-slopping.

Several studies have so far examined pH and TTA changes in pseudocereal sourdoughs. For example, [Shayesteh Kia et al. \(2024\)](#) reported that the TTA of amaranth sourdough with a dough yield of 160 stabilized at about pH 4 after seven back-slopping cycles, and no significant difference was observed between the seventh and eighth days of fermentation. Similarly, [Rouhi et al. \(2023\)](#) observed that the TTA of quinoa sourdough with a dough yield of 200 stabilized after four back-slopping cycles. Monitoring acidity stabilization during repeated back-slopping is considered an effective approach for obtaining a stable sourdough ecosystem dominated by desirable indigenous microbiota ([Sadeghi et al., 2023](#)).

3.2. Screening of predominant LAB isolates

As illustrated in [Fig. 2](#), among the three predominant LAB obtained from SASD, AS01 isolate exhibited significantly ($p < 0.05$) greater inhibitory activity against *A. flavus* than the other isolates. Therefore, this isolate was selected for further experiments as a protective starter culture for sourdough fermentation. The isolate

also showed gram-positive and catalase-negative activities, and it had no hemolytic activity.

Several studies have reported the antifungal effects of predominant LAB isolated from various sourdoughs and their application as protective starters. [Aryashad et al. \(2023\)](#) confirmed the inhibitory activity of a LAB isolate obtained from sprouted mung bean sourdough against *Aspergillus niger* and used it in controlled fermentation. [Zarali et al. \(2024\)](#) also verified the antifungal properties of a LAB strain isolated from sprouted clover sourdough and applied it as a protective starter. Generally, predominant LAB isolated from sourdough possess strong antimicrobial effects due to competition within this non-aseptic fermentation ecosystem, enabling their application as protective cultures ([Sadeghi et al., 2019](#)).

3.3. Molecular identification of the selected LAB

Successful amplification of the 1500-bp target sequence from the genomic DNA of the selected isolate was verified using agarose gel electrophoresis of the PCR amplicons ([Fig. 3](#)). Sequencing results and comparison with sequences available in the NCBI database identified the isolate as *Lactocaseibacillus casei* with 96% similarity.

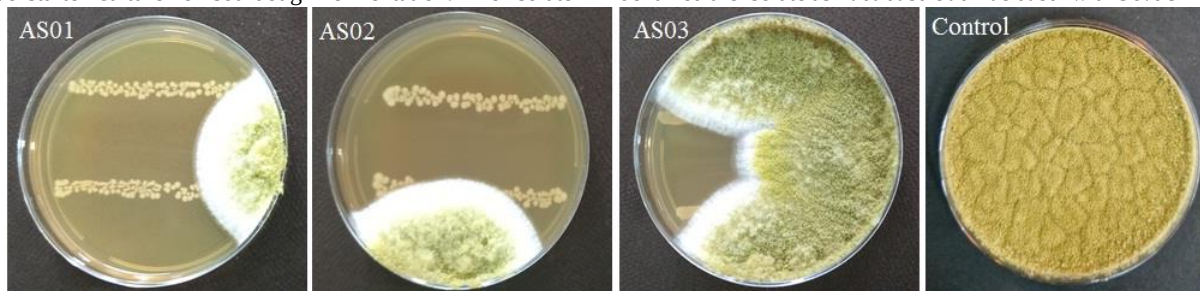


Fig. 2. Antifungal activity of the predominant lactic acid bacteria isolated from sprouted amaranth sourdough against *A. flavus* using the overlay method.

Table 1. Overall acceptability, antioxidant activity (DPPH free radical scavenging activity), surface growth of *A. flavus* and crumb pH of wheat breads containing amaranth (A), sprouted amaranth (SA), fermented sprouted amaranth (FSA) and the control sample. These data are represented as SD $\pm$ mean values, and different letters within each column indicate statistically significant differences at $p < 0.05$.

Bread sample/ characteristic	Overall acceptability	Specific volume (g/cm ³)	Antioxidant activity (%)	Surface moldiness (%)	Crumb pH
A	4.22 $\pm$ 0.15 ^b	2.69 $\pm$ 0.03 ^c	43.60 $\pm$ 3.20 ^c	15.29 $\pm$ 2.65 ^b	5.54 $\pm$ 0.03 ^a
SA	4.88 $\pm$ 0.08 ^a	3.11 $\pm$ 0.11 ^b	57.50 $\pm$ 4.40 ^b	25.47 $\pm$ 3.19 ^a	5.35 $\pm$ 0.03 ^b
FSA	4.27 $\pm$ 0.08 ^b	3.20 $\pm$ 0.05 ^b	72.90 $\pm$ 4.10 ^a	6.92 $\pm$ 1.08 ^c	5.16 $\pm$ 0.04 ^c
Control	4.33 $\pm$ 0.59 ^a	4.44 $\pm$ 0.08 ^a	32.40 $\pm$ 2.80 ^d	23.34 $\pm$ 3.77 ^a	5.33 $\pm$ 0.04 ^b

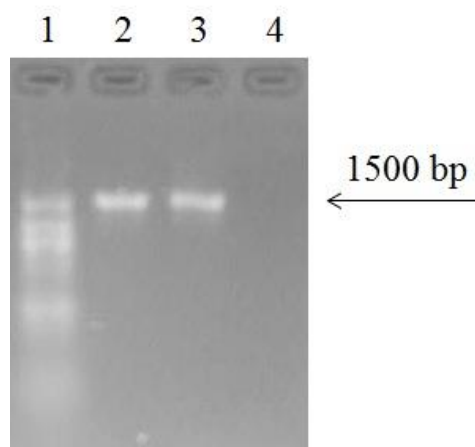


Fig. 3. Gel electrophoresis results of PCR products for the 1500 base-pair (bp) target sequence in DNA extracted from the selected lactic acid bacteria isolated from sprouted amaranth sourdough (lane 2), compared with positive and negative control samples (lanes 3 and 4, respectively) and a 50 bp DNA ladder (lane 1).

Previous studies have reported the identification of predominant LAB in pseudo-cereal and sprouted sourdoughs. For instance, [Shayesteh Kia et al. \(2024\)](#) identified *Levilactobacillus brevis* as an antifungal LAB isolated from amaranth sourdough. Likewise, *Pediococcus pentosaceus* was recently isolated from sprouted soybean sourdough and applied as a protective starter culture ([Shahryari et al., 2025](#)). The microbial biodiversity of sourdough strongly depends on substrate type, fermentation time and temperature, back-slopping frequency and dough yield. Moreover, sprouting enhances enzymatic activity and microbial diversity, increasing the probability of isolating strains with superior techno-functional properties ([Sadeghi et al., 2023](#)).

3.4. Effect of controlled sourdough fermentation on crumb hardness

As shown in [Fig. 4](#), the crumb hardness of wheat bread containing sprouted amaranth and SASD was significantly ($p < 0.05$) lower than that of other samples, whereas bread containing untreated amaranth exhibited the highest firmness. Thus, sprouting and fermentation effectively reduced the firmness of wheat bread containing amaranth. In addition, the specific volume of bread containing sourdough was higher than that of bread with only sprouted amaranth ([Table 1](#)), indicating improved textural properties.

[Shahryari et al. \(2025\)](#) reported that controlled fermentation of sprouted soybean sourdough significantly reduced crumb firmness compared with breads containing soybean or sprouted soybean

alone. Similarly, [Ziaee rizi et al. \(2024\)](#) observed the lowest firmness and highest specific volume in wheat bread containing sprouted mung bean sourdough. Although sprouted grains may soften texture due to increased enzymatic activity, they may reduce specific volume, which is undesirable. Therefore, controlled sourdough fermentation represents an effective strategy for improving the texture of breads enriched with sprouted legumes or pseudo-cereals ([Gobbetti et al., 2019](#)).

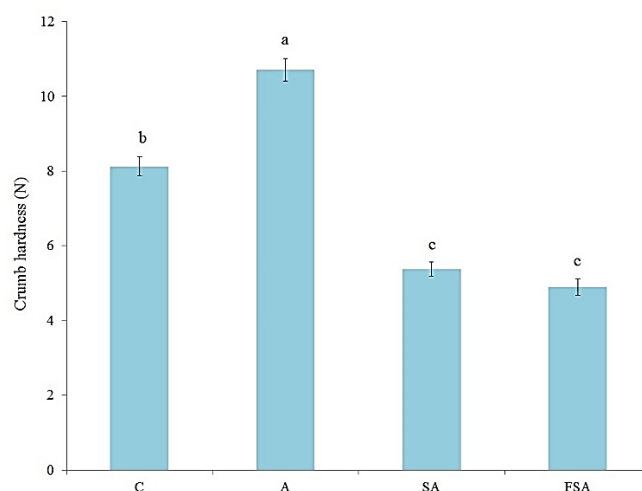


Fig. 4. Crumb hardness of wheat breads containing amaranth (A), sprouted amaranth (SA), fermented sprouted amaranth (FSA) and the control sample (C). Different letters indicate statistically significant differences at $p < 0.05$.

3.5. Effect of controlled sourdough fermentation on overall acceptability

Overall acceptability scores are shown in [Table 1](#). The bread containing sprouted amaranth did not differ significantly ($p > 0.05$) from the control sample. Furthermore, although breads containing sourdough or amaranth showed slightly lower acceptability than the control, the differences were not statistically significant.

According to [Aryashad et al. \(2023\)](#), the overall acceptability of wheat bread containing sprouted mung bean sourdough showed no significant difference compared with the control sample. Similarly, [Purabdollah et al. \(2020\)](#) reported that the use of acorn sourdough in wheat bread did not result in a significant difference in overall acceptability compared with the control. However, controlled sourdough fermentation has been shown to improve the aromatic compound profile ([Shahryari et al., 2025](#); [Sadeghi et al., 2026](#)).



Fig. 5. The extent of *A. flavus* growth on the surface of wheat breads containing amaranth (A), sprouted amaranth (SA), fermented sprouted amaranth (FSA) and the control sample (C) after 5 days of storage period at room temperature.

Sensory properties of sourdough bread depend on various factors, including substrate type, starter culture and fermentation conditions. LAB strains contribute to aroma and flavor development through the production of volatile compounds and flavor precursors. Moreover, improved gas retention and crumb structure enhance mouth-feel and chewiness. Acidic conditions during fermentation may also promote Maillard reactions during baking, influencing crust color and consumer acceptance (Sadeghi et al., 2023).

3.6. Effect of controlled sourdough fermentation on the reduction of mold growth

Surface growth of *A. flavus* on the produced bread samples is presented in Table 1 and Fig. 5. Accordingly, bread containing SASD exhibited significantly ($p < 0.05$) lower mold growth than the other samples. Interestingly, sprouting alone increased fungal growth, whereas combining sprouting with fermentation effectively prevented mold development.

The results of the study by Rouhi et al. (2023) showed that controlled fermentation of quinoa sourdough had significantly lower surface mold growth and the highest antifungal effect compared with the other produced samples. In the study by Sadeghi et al. (2024b), the effectiveness of controlled fermentation of barley sourdough in preventing surface mold growth on the produced breads was also confirmed. Aryashad et al. (2023) likewise reported that surface mold growth in wheat bread containing sprouted mung bean sourdough was significantly lower than in samples containing mung bean and sprouted mung bean. This protective effect is attributed to antifungal metabolites, including organic acids, that are produced during controlled fermentation. Considering the growing demand for clean-label products and reduced use of synthetic preservatives, controlled sourdough fermentation offers a natural and effective method for delaying mold spoilage in bakery products (Sadeghi et al., 2023).

3.7. Effect of controlled sourdough fermentation on antioxidant activity

As indicated in Table 1, wheat bread containing SASD showed significantly ($p < 0.05$) higher antioxidant activity than all other samples. Breads containing sprouted amaranth or untreated amaranth also exhibited greater antioxidant capacity than the control.

According to the results of Zarali et al. (2024), the methanolic extract of wheat bread containing clover seed flour exhibited the highest DPPH free radical scavenging activity compared with the other breads produced. However, the antioxidant activity of this

sample did not differ significantly from that of bread containing spontaneously fermented germinated clover. In addition, the water/salt-soluble extract of bread containing controlled fermented germinated clover showed the highest antioxidant activity compared with the water/salt extracts of the other produced samples. Shahryari et al. (2025) reported that the antioxidant activity of both the methanolic extract and the water/salt-soluble extract from wheat bread containing sprouted soybean sourdough was significantly higher than that of breads containing either soybean or sprouted soybean alone. In addition, fermentation may generate phenolic compounds and antioxidant peptides, further enhancing antioxidant potential (Purabdolah et al., 2020).

3.8. Survival of probiotic culture after baking

Probiotic survival results are shown in Fig. 6. The survival of *B. coagulans* in bread containing SASD (FSA+PRO) was significantly higher ($p < 0.05$) than in bread without sourdough (C+PRO), which was used as positive control in this assay. These findings confirm the prebiotic effect of sprouted amaranth in enhancing probiotic survival during processing. Moreover, negative control (C) and FSA-added samples were not containing viable cells after baking.

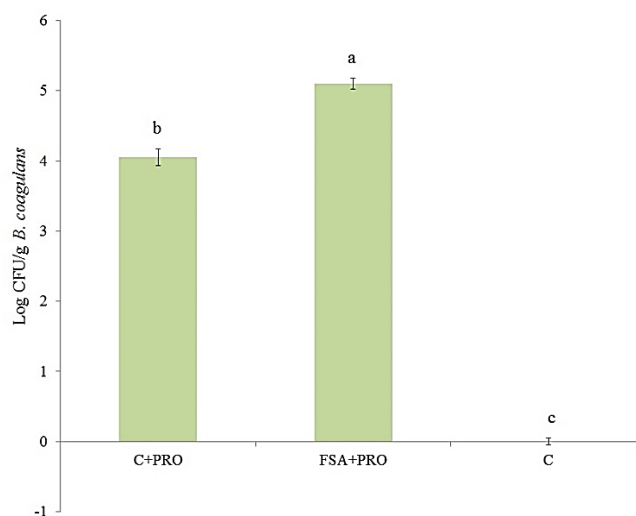


Fig. 6. The viability of the probiotic *B. coagulans* in wheat breads containing fermented sprouted amaranth (FSA+PRO) and without this sourdough (C+PRO; as positive control), compared with the control sample without probiotic culture (C; as negative control). Different letters indicate statistically significant differences at $p < 0.05$.

There are reports regarding the favorable viability of various *Bacillus* species after baking of bakery products. For example, in the study by Soares et al. (2019), the viability of the investigated *Bacillus* strains in bread was considerably higher than that of other probiotics. Ganjouri et al. (2012) reported that *B. coagulans* counts decreased from 10^8 to 10^6 CFU/g after baking but still remained viable. The survival of probiotics during baking is a major challenge in probiotic bakery products; therefore, thermotolerant spore-forming bacteria such as *B. coagulans* are preferred due to their superior industrial applicability compared with encapsulation or edible coating techniques. After passing through the stomach, the spores germinate and exert beneficial effects in the intestine (Sadeghi et al., 2024a).

4. Conclusion

This study demonstrated that controlled fermentation of SASD is an effective strategy for improving the techno-functional and storage stability characteristics of synbiotic wheat bread. The TTA of the sourdough stabilized after successive back-slopping cycles, enabling the isolation of predominant LAB with desirable protective properties. Among the isolates, *L. casei* showed superior antifungal activity against *A. flavus* and was successfully applied as a starter culture for controlled sourdough fermentation. Incorporation of the SASD significantly enhanced bread quality by reducing crumb hardness, increasing specific volume, improving antioxidant activity and inhibiting surface fungal growth during storage. Furthermore, the presence of sprouted amaranth components improved the post-baking survival of the probiotic *B. coagulans*, confirming the synbiotic functionality of the developed bread. Overall, the combined processes of sprouting and controlled sourdough fermentation provided synergistic effects that improved textural properties, extended shelf life, enhanced bioactive potential and supported probiotic viability. Therefore, the application of SASD can be considered a promising clean-label and natural approach for the development of functional and synbiotic wheat bread with added health benefits.

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Conflict of interest

The authors declare that there is no conflict of interest.

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