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Impact of controlled fermentation on quality characteristics and antioxidant activity of sourdough bread

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ABSTRACT

As consumer demand for clean-label bread and natural ingredients continues to rise, bio-preservation methods like sourdough fermentation are gaining attention for their potential to enhance both the quality and shelf-life of this staple food to reduce the need for chemical preservatives. This study explores the effects of sourdough fermentation and dough formulation on the quality attributes of wheat bread. Using a starter culture, we compared the textural properties, overall acceptance, shelf-life, and antioxidant activity of sourdough bread with 1% and 10% sugar contents to bread made with chemically acidified dough using lactic and acetic acids at similar sugar levels. Bread with 1% sugar and chemically acidified dough (CA-0.9) exhibited the lowest crumb chewiness, significantly differing ($p < 0.05$) from other samples except for chemically acidified bread with propionic acid and 1% sugar (CA-PA-0.9). The highest porosity was observed in bread with 1% sugar and sourdough (SD-0.9) as well as in CA-0.9. Although the bread with sourdough and 10% sugar (SD-9) had the highest chewiness, it achieved the highest overall acceptance score due to flavour enhancement by the sugar. Bread containing propionic acid and 10% sugar showed the longest shelf-life, with no significant difference from SD-9. The DPPH radical scavenging activity was also 33.02% for CA-9 and 23.72% for SD-9. Accordingly, controlled fermentation with sourdough and 10% sugar significantly enhanced the quality attributes of wheat bread, offering potential as a natural bio preservative and aligning with the growing consumer demand for clean-label products in the baking industry.

Keywords: Sourdough starter culture; Texture profile; Overall acceptance; Shelf-life; Antioxidant activity.

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1. Introduction

Sourdough fermentation is a traditional process widely used in the production of wheat and rye bread. Although the industry largely replaced sourdough fermentation with yeast fermentation over the last century, there has been renewed interest in controlled sourdough fermentation using starter cultures. This renewed interest is due to the proven benefits of sourdough in improving both the sensory and nutritional qualities of bread, as well as extending its shelf-life (Gobbetti et al., 2019). Despite the availability of various dough enhancers, sourdough continues to play a significant role in baking, particularly in response to the growing market demand for functional products and the unique health benefits provided by microbial metabolites produced during sourdough fermentation (Sadeghi et al., 2023). Sourdough fermentation is recognized as an effective method for enhancing the quality characteristics of wheat bread. The fermentation conditions, which influence the content and composition of metabolites such as organic acids, directly impact the properties of sourdough bread (Sadeghi et al., 2023). Controlled sourdough fermentation with starter cultures has been demonstrated to improve various aspects of wheat bread, including textural properties (Aryashad et al., 2023; Ebrahimi et al., 2022), overall acceptance (Ebrahimi et al., 2020; Pahlavani et al., 2024), shelf-life (Rouhi et al., 2023; Sadeghi et al., 2019), and antioxidant activity (Hajinia et al., 2021; Purabdolah et al., 2020). While various lactic acid bacteria have been isolated from sourdough, only a few strains,

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such as *Fructilactobacillus sanfranciscensis*, *Lactiplantibacillus plantarum*, *Limosilactobacillus pontis*, and *Lactobacillus rossiae*, are typically dominant in industrial fermentations. Among these, *F. sanfranciscensis* significantly influences bread characteristics, including texture, aroma, and flavor, and increases the bioavailability of minerals and bioactive compounds (Rogalski et al., 2021).

Sourdough also enhances bread's porosity and texture consistency while reducing its hardness, gumminess, and chewiness, which are positively related to overall bread quality (Wronkowska et al., 2015). However, the role of sourdough in bread volume is complex; excessive sourdough acidification can degrade the gluten network, reducing gas retention capacity and resulting in decreased bread volume (Verdonck et al., 2023; Xu et al., 2018). Conversely, sourdough can improve dough rheological properties and create a more suitable environment for yeast activity, which may enhance bread volume under certain conditions (Cakir et al., 2021; Crowley et al., 2013). The impact of sourdough on bread volume is influenced by several factors, including the type and amount of sourdough used, the starter culture, flour composition, fermentation conditions, and baking process. Further research is needed to optimize sourdough conditions in bread production. Lactic and acetic acids produced by lactic acid bacteria during sourdough fermentation contribute not only to microbiological shelf-life extension but also to dough leavening, acidification, and improved bread quality (Axel et al., 2016; Gänzle, 2015; Gobbetti et al., 2014). The improvement in volume and shelf-life of sourdough bread largely depends on the nature and intensity of the acidification process (Clarke et al., 2003).

Several factors, including bread formulation, flour extraction percentage, baking time, and temperature, influence the antioxidant capacity of bread (Lindenmeier and Hofmann, 2004; Michalska et al., 2008). The Maillard reaction, which occurs between amino groups of proteins and carbonyl compounds of reducing sugars, also plays a crucial role. This reaction enhances the product's quality attributes and produces aromatic compounds with antioxidant properties such as radical scavenging and metal chelating abilities (Bastos et al., 2012; Echavarría et al., 2012; Hwang et al., 2011). Shen et al. (2018) reported that increasing sucrose or fructose levels generally balanced bread volume and antioxidant capacity. Additionally, high glucose levels during fermentation enhance the activity of antioxidant-related enzymes in yeast (Xie et al., 2022).

The quality characteristics of bread made with sourdough fermented using *F. sanfranciscensis* compared to chemically acidified dough have not yet been extensively explored within the baking industry. Therefore, this study aims to investigate the effects of sourdough, varying sugar levels, and propionic acid on key attributes of wheat bread, such as texture, overall consumer acceptance, shelf-life, and antioxidant activity. The research focuses on the potential of sourdough as a natural preservative, aiming to reduce the need for chemical additives like propionic acid. By understanding how these factors influence bread quality and preservation, the study seeks to provide valuable insights that could lead to the production of wheat bread with enhanced nutritional and sensory properties, longer shelf-life, and greater appeal to health-conscious consumers.

2. Material and Methods

2.1. Starter culture and chemicals

The *F. sanfranciscensis* 20451 was obtained from the Microbial Bank of the University of Alberta (Canada) thanks to the Prof. M. Ganzle. Wheat flour, baker's yeast, salt, sunflower oil, and sugar were purchased from local stores. The chemicals used included diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, Tris-HCl, and methanol for antioxidant assays and microbial culture media, which were sourced from Merck (Germany) and Liofilchem (Italy).

2.2. Controlled sourdough fermentation

For controlled sourdough fermentation, a 24-hour culture of *F. sanfranciscensis* was centrifuged at 5000 g for 10 minutes at 4 °C and washed twice. The resulting cell pellet was suspended in a sterile Ringer solution. This microbial suspension was then mixed with wheat flour at a 1:1 (w/w) ratio. After 24 hour fermentation at 30 °C, a one-time inoculation process was conducted by mixing 10% of the previous sourdough with equal amounts of flour and water, achieving a dough yield (the ratio of dough to flour) of 200. After 24 hours, the bacterial population in the sourdough reached 10^9-10^{10} CFU/g, and the pH was between 3.6 and 3.8. Additionally, a chemically acidified sample was prepared by combining equal parts of water and flour with lactic acid/acetic acid in a 1:4 ratio to achieve a pH of 3.6 to 3.8. The concentrations of lactic acid and acetic acid used were 85% and 99%, respectively (Clarke et al., 2004).

2.3. Bread processing

2.3.1. Dough preparing

To prepare the bread dough, a blend of wheat flour (70% extraction rate, 12% moisture, 0.45% ash, 1% fiber, and 10% protein), water, baker's yeast, salt, oil, sugar, and either sourdough made with *F. sanfranciscensis* or chemically acidified dough was used. The specific quantities of these ingredients are detailed in Table 1. In total, eight varieties of wheat bread were produced, including sourdough with 10% sugar (SD-9), sourdough with 1% sugar (SD-0.9), sourdough + propionic acid with 10% sugar (SD-PA-9), sourdough + propionic acid with 1% sugar (SD-PA-0.9), chemically acidified dough with 10% sugar (CA-9), chemically acidified dough with 1% sugar (CA-0.9), chemically acidified dough + propionic acid with 10% sugar (CA-PA-9), and chemically acidified dough + propionic acid with 1% sugar (CA-PA-0.9). The bread was produced according to the protocol of Black et al. (2013). The ingredients were mixed for one minute at low speed and six minutes at high speed using a KitchenAid mixer (USA). The resulting dough was shaped and proofed in 6 × 9 × 18 cm bread pans for one hour at 32 °C. Propionic acid (0.1 g) was added to the dough just before baking to minimize its inhibitory effect on yeast activity. The sugar levels used were also adjusted for flavor and to regulate the water activity of the samples, and this formulation is commonly used in some countries.

2.3.2. Bread manufacturing

After proofing, the dough was baked at 175 °C for 25 minutes in a GE Appliances hot air oven (USA) and then allowed to cool on wire racks for two hours before further testing.

Table 1. Formulations of the produced bread samples.

	Chemically acidified dough				Sourdough			
Ingredients (g)	**CA-9**	**CA-0.9**	**CA-PA-9**	**CA-PA-0.9**	**SD-9**	**SD-0.9**	**SD-PA-9**	**SD-PA-0.9**
Wheat flour	90	90	90	90	90	90	90	90
Water	50	50	50	50	50	50	50	50
Baker's Yeast	2	2	2	2	2	2	2	2
Salt	2	2	2	2	2	2	2	2
Canola oil	2	2	2	2	2	2	2	2
Sugar	9	0.9	9	0.9	9	0.9	9	0.9
Controlled sourdough	-	-	-	-	20	20	20	20
Chemically acidified dough	20	20	20	20	-	-	-	-
Propionic acid	-	-	0.926	0.926	-	-	0.926	0.926

2.4. Determination of pH and water activity

The pH of the produced bread was measured using a pH meter (FisherScientific, USA), and their water activity was measured using an a_w meter (AQUALAB, USA) at 20 °C.

2.5. Evaluation of textural properties

Texture profile analysis (TPA) of the bread was conducted two hours after baking. A cylindrical aluminium probe (25 mm in length) was used to apply compression at a rate of 0.1 mm/s to achieve 50% compression on cubic bread samples (Katina et al., 2006). Textural properties such as chewiness, cohesiveness, and gumminess were determined using a texture analyser (Stable Microsystem, UK). Bread porosity was assessed using image analysis with ImageJ software, version 1.42e (Hajinia et al., 2021). Images of bread slices were captured with an iPhone 13 against a black background under room lighting. The images were imported into ImageJ software, converted to grayscale, and thresholded to distinguish air cells from the bread's solid structure. The images were then converted to binary format, allowing the analyze particles function to quantify the area occupied by air cells. Porosity was calculated as the ratio of the total area of air cells to the total area of the bread slice.

2.6. Evaluation of overall acceptance

The overall acceptance of the produced bread (based on taste, aroma, color, mouthfeel, and chewability) was evaluated by a panel of trained individuals using a five-point hedonic scale. A score of 5 indicated the highest acceptance, while a score of 1 indicated the lowest acceptance (Purabdolah et al., 2020).

2.7. Determination of shelf-life without fungal contamination

The shelf-life of the produced bread without fungal contamination (mold-free) was determined using a modified method from Gerez et al. (2009). Bread slices were stored in sterile polyethylene bags at room temperature until the first signs of fungal growth were observed (daily imaging and analysis using ImageJ software).

2.8. Determination of antioxidant activity

Antioxidant activity was evaluated using the DPPH radical scavenging assay (Ainsworth and Gillespie, 2007), while total phenolic content was measured by the Folin-Ciocalteu method (Sharma and Bhat, 2009), according to established protocols. Methanolic extracts (10% w/v) were prepared in 80% methanol, and aqueous extracts (25% w/v) were prepared in 50 mM Tris-HCl. Absorbance was measured at 517 nm using a spectrophotometer (PGI, UK). Antioxidant activity and phenolic content as gallic acid equivalents (mg/g) were expressed compared to the standard curve.

2.9. Statistical Analysis

Statistical analysis of textural properties, overall acceptance, shelf-life, and antioxidant activity of the bread samples was performed using a completely randomized design and one-way ANOVA. All experiments were conducted in triplicate except the antioxidant test that were done in duplicate, and results were analysed using Duncan's multiple range test for significance at $p< 0.05$ using SAS software, version 9.1.

3. Results and Discussion

3.1. Physicochemical characteristics of the sourdough and bread

The general characteristics of sourdough, chemically acidified dough, and bread produced with 1% and 10% sugar are presented in Table 2. The pH of the produced bread ranged from 5.21 to 5.29. Previous studies have reported the pH of chemically acidified bread to be between 3.8 and 5.7, depending on the acetic and lactic acid content (Debonne et al., 2020). The highest pH values in Debonne et al. (2020) align with the results of this study, indicating that our bread samples fall within the upper range of chemically acidified bread. A similar range of pH (4.5 to 5.5) has been reported by Corsetti and Settanni (2007) in their study on sourdough fermentation, further supporting the consistency of our results with those typically observed in sourdough bread production. Another study indicated that the desirable pH range for sourdough bread is between 5 and 5.5 (Gerez et al., 2009), which closely matches the findings of the current study. This alignment suggests that the sourdough bread in our study achieved a pH level conducive to desirable sensory and preservation qualities. Moreover, these pH levels are known to inhibit the growth of spoilage organisms, thus enhancing the microbiological safety of the bread (Gobbetti et al., 2005).

Table 2. Characteristics of sourdough, chemically acidified dough and manufactured bread.

Characteristics	10% sugar	1% sugar
Dough yield*	200	
pH of sourdough or chemically acidified dough	3.8 ± 0.2	
LAB of sourdough (CFU g^{-1})	10^9-10^{10}	
pH of bread	5.21 ± 0.20	5.29 ± 0.15
a_w of bread	0.96 ± 0.01	0.94 ± 0.01

*The ratio of flour to water determines the thickness of the sourdough and can be expressed as dough yield (DY), defined as 100 parts of flour plus the amount of water (in parts) used for hydration (Katina et al., 2014).

Additionally, the water activity (a_w) in bread with 10% sugar was lower than in bread with 1% sugar, with values of 0.94 and 0.96, respectively. These findings are consistent with previous research, which reported the water activity of chemically acidified bread to be 0.967 ± 0.003 (Debonne et al., 2020). The reduction in water activity with higher sugar content is likely due to sugar's hygroscopic properties, which reduce free water in the product. It has been reported that water activity values of bread are not significantly affected by acid content; however, both pH and water activity play critical roles in influencing the fermentation capacity and, subsequently, the texture of the produced bread (Cauvain, 2015; Debonne et al., 2020). Lower water activity combined with optimal pH levels can significantly enhance the textural properties and overall stability of bread, making these factors critical in the formulation of high-quality baked products (Corsetti & Settanni, 2007). Arendt et al. (2007) categorized the impact of acidification on dough properties into three main categories: (1) direct effects on dough structure, such as gluten swelling in the presence of acid and increased solubility of gluten proteins at acidic pH, leading to reduced dough stability and extensibility; (2) effects of acid on enzymes present in grains; and (3) effects on the activity of microorganisms and baker's yeast. These interactions underscore the complexity of balancing pH and water activity to optimize both the technological and sensory attributes of bread (Clarke et al., 2004).

3.2. Texture characteristics of the produced bread

Table 3 shows the texture characteristics of the produced bread. According to the results, SD-PA-9 had the highest chewiness (504.80 ± 65.90), followed by CA-PA-9 and SD-9. It seems that increasing the sugar content in the bread formulation, along with reduced water activity and increased gumminess, had the greatest impact on increasing hardness. This is because these two samples had the lowest water activity (0.94), which was significantly different ($p < 0.05$) from other samples. Conversely, the lowest chewiness was noted in the CA-0.9 with and CA-PA-0.9 samples, both containing 1% sugar and the highest water activity. The SD-9 bread had significantly different and lower gumminess compared to other samples. Breads with 10% sugar, due to their increased hardness, exhibited lower gumminess. Generally, there is an inverse relationship between bread hardness and gumminess; as hardness increases, gumminess decreases. Harder bread typically has a denser structure, resulting in less elasticity and reduced ability to return to its original shape after compression. In contrast, softer bread (less hardness) has a more porous structure, allowing it to be more flexible and elastic (Torbica et al., 2008). Moreover, samples with 1% sugar (SD-0.9 and CA-0.9) had the highest porosity. Adding propionic acid to both sourdough and chemically acidified bread groups reduced porosity, likely due to its negative effect on yeast activity and reduced gas production (Fig. 1).

Fig. 1. Cross-section image of the crumb of manufactured bread. The aforementioned codes indicate produced bread including SD-9: sourdough 10% sugar, SD-0.9: sourdough 1% sugar, SD-PA-9: sourdough + propionic acid 10% sugar, SD-PA-0.9: sourdough + propionic acid 1% sugar, CA-9: chemically acidified 10% sugar, CA-0.9: chemically acidified 1% sugar, CA-PA-9: chemically acidified + propionic acid 10% sugar and CA-PA-0.9: chemically acidified + propionic acid 1% sugar.

Shen et al. (2018) reported that doubling the sugar content in the bread formulation increased hardness and reduced volume. Another study investigated the effects of adding inulin (a type of carbohydrate) on bread texture properties, finding that the hardness and specific volume of the produced bread ranged from 765.09-3189.11 g and 1.37-2.65 cm^3/g, respectively. Texture characteristics such as chewiness and gumminess are critical for both consumer satisfaction and industrial production. Softer bread with less gumminess is generally preferred by consumers, as it is easier to chew and perceived as fresher. In industrial settings, controlling these texture attributes is essential for maintaining product quality and shelf-life. Excessive chewiness can indicate staling, while high gumminess can lead to a less enjoyable eating experience. The inclusion of sourdough in bread formulation can help optimize these textural properties, enhancing consumer appeal and extending shelf-life through natural preservation methods, as highlighted by Casado et al. (2017). Mollakhalili-Meybodi et al. (2023) found that a higher carbohydrate content, such as inulin, led to a firmer texture and reduced loaf volume due to interference with gluten network formation and gas retention during fermentation. The increased sugar content also contributed to a denser crumb structure, further enhancing the bread's hardness. These findings are in agreement with the results of the present study. Tomić et al. (2023) also examined the physicochemical properties of bread with varying percentages of sourdough and reported a gumminess of 0.947 ± 0.008 cm in samples with 25% sourdough. Additionally, Debonne et al. (2020) reported that the hardness of bread containing 50 mmol/kg of acetic acid was three times greater than that of the control sample. Another factor affecting hardness is the use of sourdough or chemical acidification. According to Corsetti et al. (2000), chemically acidified bread had lower hardness and greater volume during 24 hours of storage, but it staled faster compared to sourdough bread. In a different study, researchers found that adding organic acids and phenolic compounds to bread could increase the softness of the bread (Su et al., 2019; Xu et al., 2019). These researchers attributed this effect to the rapid chemical acidification and the provision of optimal conditions for yeast metabolism.

Table 3. Quality characteristics of manufactured bread samples.

Sample code	Chewiness (gr.cm)	Springiness	Gumminess	Porosity (%)	Overall acceptability	Mold-free shelf-life (day)
SD-9	424.30 ± 68.01[ab]	0.82 ± 0.03[d]	519.06 ± 84.53[ab]	8.95 ± 2.61[ab]	4.6 ± 0.55[a]	6 ± 0[a]
SD-0.9	368.20 ± 59.90[b]	0.85 ± 0.01[bcd]	431.19 ± 72.31[bc]	10.90 ± 2.40[a]	4.2 ± 0.45[ab]	4 ± 0[b]
SD-PA-9	504.80 ± 65.90[a]	0.86 ± 0.01[abcd]	584.03 ± 69.52[a]	9.25 ± 0.35[ab]	3.6 ± 0.55[bc]	7 ± 0[a]
SD-PA-0.9	421.23 ± 75.22[ab]	0.90 ± 0.02[ab]	469.20 ± 87.44[abc]	7.85 ± 1.91[ab]	3.6 ± 0.55[bc]	6 ± 0[a]
CA-9	356.59 ± 52.34[b]	0.83 ± 0.04[cd]	425.95 ± 44.37[bc]	7.30 ± 0.14[b]	4.2 ± 0.84[ab]	6 ± 0[a]
CA-0.9	223.60 ± 24.40[c]	0.88 ± 0.02[abc]	252.96 ± 25.43[d]	10.90 ± 2.62[a]	3.2 ± 1.10[c]	4 ± 0[b]
CA-PA-9	432.73 ± 75.67[ab]	0.87 ± 0.04[abc]	496.33 ± 70.32[ab]	9.23 ± 2.60[ab]	3.4 ± 0.55[bc]	7 ± 0[a]
CA-PA-0.9	320.08 ± 57.97[bc]	0.91 ± 0.02[a]	353.52 ± 62.53[cd]	6.80 ± 1.41[ab]	3.4 ± 0.55[bc]	6 ± 0[a]

The same letter in each column indicates the absence of a significant difference at $p > 0.05$. SD-9: sourdough 10% sugar, SD-0.9: sourdough 1% sugar, SD-PA-9: sourdough + propionic acid 10% sugar, SD-PA-0.9: sourdough + propionic acid 1% sugar, CA-9: chemically acidified 10% sugar, CA-0.9: chemically acidified 1% sugar, CA-PA-9: chemically acidified + propionic acid 10% sugar and CA-PA-0.9: chemically acidified + propionic acid 1% sugar.

3.3. Sensory acceptance

The results in Table 3 show significant sensory differences among the various sourdough and chemically acidified bread samples, which can be attributed to the starter culture activity and its produced metabolites. The SD-9 bread, which contained the highest amount of sugar, received the highest overall acceptance score despite having the greatest hardness. This trend was also observed in the bread made with chemically acidified dough. These results indicate that sugar can mask the sour taste of sourdough or organic acids, thereby moderating the flavor and aroma of the produced bread. Additionally, sugar can enhance the activity of lactic acid bacteria and yeasts, leading to the production of more aromatic compounds that positively affect the sensory acceptance of the bread (Jekle et al., 2010).

On the other hand, samples containing propionic acid, such as SD-PA-9 and CA-PA-9, had lower overall acceptance scores, indicating the negative effect of propionate on the sensory properties of the produced bread. While this compound is commonly used as a preservative in the baking industry, it appears to affect yeast activity, thereby impacting the taste and aroma of the bread and reducing its overall acceptance by consumers. In this regard, Carrillo-Meza et al. (2016) reported that propionic acid often imparts an off-flavor, described as sour or bitter, and negatively affects the bread's flavour profile, although the sensory impact was not always significant depending on the concentration used. Accordingly, application of sourdough as a bio-preservative and increasing the sugar content in the formulation, which helps extend the shelf-life of bread, can lead to a reduction in the concentration of propionic acid, thereby improving sensory acceptance. The positive impact of controlled sourdough fermentation on the sensory properties of produced wheat bread has been confirmed in several studies (Ebrahimi et al., 2022; Pahlavani et al., 2024). The production of aroma compounds and flavor precursors by the microbial flora of sourdough significantly improves the overall acceptance of the produced bread (Sadeghi et al., 2023). It has also been reported that during sourdough fermentation, increased proteolytic activity leads to the production of more peptides, and the reaction between carbohydrates and amino acids at the high temperatures of baking intensifies the Maillard reaction, ultimately improving the color of the produced bread. The results of the present study align with these observations, as the SD-9 sample, which contained more sugar, likely showed improved color due to the increased activity of *F. sanfranciscensis* and heightened proteolytic activity during fermentation (Gänzle, 2014; Torrieri et al., 2014).

3.4. Mold-free shelf-life of the produced bread

As shown in Table 3, samples containing propionic acid with higher sugar content (SD-9 and CA-9) had a longer shelf-life without mold growth. This indicates that, in addition to the inhibitory role of propionic acid, the reduction of water activity by sugar also plays a significant role. Interestingly, the shelf-life of the sourdough bread with 10% sugar (SD-9) was not significantly different ($p > 0.05$) from these samples. Thus, sourdough fermentation can be used as a bio-preservative in bread processing. Generally, delaying mold growth in bread is a much more critical factor in evaluating the shelf-life of this product than its staling, as it has a direct impact on consumer health. The role of controlled sourdough fermentation in delaying mold growth in wheat bread has been demonstrated in numerous studies (Rouhi et al., 2023; Sadeghi et al., 2019). It has also been reported that the reduction of pH due to the production of organic acids, along with other antifungal metabolites produced by lactic acid bacteria, plays a crucial role in this effect (Sadeghi et al., 2023). Recently, Zarali et al. (2024), Ziaee rizi et al. (2024), and Shayesteh Kia et al. (2024) highlighted the importance of using controlled sourdough fermentation to delay mold growth in bread, which benefits both the economy and consumer health. Ziaee rizi et al. (2024) found that bread made with *Levilactobacillus brevis* sourdough and ginger extract remained mold-free for 4 days, similar to the SD-0.9 bread in this study. Shayesteh Kia et al. (2024) also observed the least mold (35%) on bread with amaranth sourdough after 7 days. However, in this study, mold growth on SD-PA-9 bread only began after 7 days. This difference might be due to the fungal strains used in their study, as this study focused on testing the bread's shelf-life without introducing contaminants. During controlled sourdough fermentation, the production of a wide range of inhibitory metabolites such as short-chain fatty acids, cyclic fatty acids, antifungal peptides, antioxidant compounds, organic acids, and their derivatives prevents mold development in the produced bread. Based on the findings of this study, the simultaneous application of controlled sourdough fermentation and sugar in the bread formulation can be significant in controlling mold growth by enabling the combined effect of produced antifungal metabolites and reducing the product's water activity.

3.5. Antioxidant activity of the produced bread

Fig. 2 shows the antioxidant activity and phenolic content of the produced bread. The highest DPPH radical scavenging activity was observed in the CA-9 sample (33.029 ± 0.28%) followed by the SD-9 bread (23.72 ± 0.08%), with a significant difference between them ($p < 0.05$). Additionally, bread with higher sugar content (10%) exhibited greater DPPH radical scavenging activity, although the antioxidant activity of sourdough and chemically acidified bread did not show a consistent trend. The presence of propionate in the bread composition also reduced its antioxidant activity. Furthermore, the total phenolic content in chemically acidified bread was higher than those of the sourdough bread, and bread with higher sugar content had higher total phenolic content.

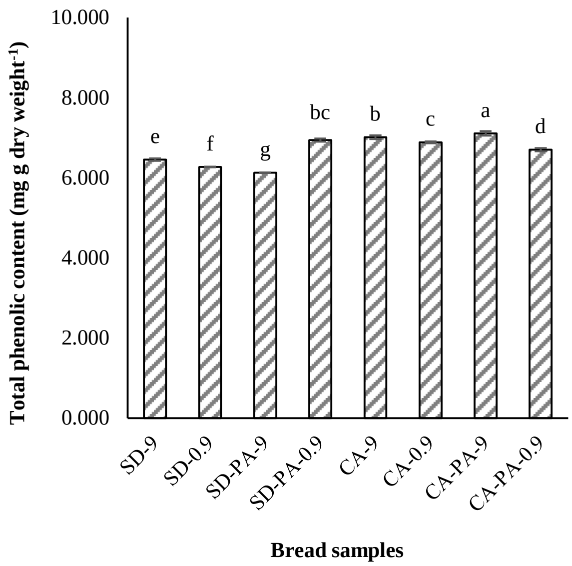

Fig. 2. Antioxidant activity (DPPH free radical scavenging) and total phenolic content (mg g^{-1}) of manufactured bread (the upper and the lower pictures, respectively). SD-9: sourdough 10% sugar, SD-0.9: sourdough 1% sugar, SD-PA-9: sourdough + propionic acid 10% sugar, SD-PA-0.9: sourdough + propionic acid 1% sugar, CA-9: chemically acidified 10% sugar, CA-0.9: chemically acidified 1% sugar, CA-PA-9: chemically acidified + propionic acid 10% sugar and CA-PA-0.9: chemically acidified + propionic acid 1% sugar. Different letters indicate significant differences ($p < 0.05$).

The observed antioxidant activity in bread with higher sugar content may be due to the production of compounds resulting from increased microbial activity. Generally, the DPPH radical scavenging activity assay measures the ability of antioxidant compounds in the sample to neutralize free radicals, while the Folin-Ciocalteu method provides the total phenolic content in the sample (Abramovič et al., 2018). Similarly, Shen et al. (2018) reported that the DPPH radical scavenging activity in the crumb of bread was higher than in the crust, ranging from 1.29 to 23.73 μmol L^{-1}. These researchers attributed the antioxidant activity to the increase in reducing sugars during fermentation, which enhances the Maillard reaction. Other researchers have also shown that fermentation leads to the production of peptides with antioxidant activity that are not affected by the baking process (Galli et al., 2019; Luti et al., 2021).

Another study (Seis Subaşı and Ercan, 2023) reported that the phenolic content and antioxidant activity of sourdough containing *F. sanfranciscensis* were higher than those of other starter cultures used. Hajinia et al. (2021) reported that the use of sourdough fermented with *Pediococcus pentosaceus* improved the antioxidant activity of the produced bread compared to the control sample. They stated that sourdough fermentation stimulates the enzymatic activity of the fermented grains, leading to the breakdown of substances into more soluble and simpler forms. During the fermentation process, not only do the solubility and digestibility of proteins increase, but existing proteins are also converted into various peptides and free amino acids. The antioxidant activity of these peptides depends on their amino acid composition, structure, and hydrophobic properties. Other researchers have attributed the antioxidant activity in sourdough bread to the production of phenolic compounds and bioactive peptides by lactic acid bacteria during the fermentation process, and have noted that the baking process may reduce the antioxidant activity to some extent (Purabdolah et al., 2020).

4. Conclusion

In this study, the effect of adding sugar and propionic acid to the formulation of sourdough bread containing *F. sanfranciscensis* on the textural properties, overall acceptability, shelf-life, and antioxidant activity of leavened wheat bread was investigated. The results showed that the use of sourdough and chemically acidified dough with various sugar levels significantly influenced the quality characteristics of the produced bread. Bread made with 1% sugar exhibited less chewiness, whereas increasing the sugar content led to increased chewiness and decreased porosity. Although the addition of propionic acid extended the shelf-life of the produced bread, it had a negative impact on the textural properties and overall acceptability due to its adverse effect on yeast activity. Overall, sourdough-containing samples showed better overall acceptability compared to their chemically acidified counterparts due to the controlled fermentation by the starter culture used. The SD-9 bread, despite having the highest hardness, received the highest overall acceptability score due to its sugar content and moderated sour taste. Additionally, the shelf-life of this sample without mold growth did not significantly differ from samples containing propionic acid. This bread also exhibited favourable antioxidant activity compared to other samples. Considering these characteristics, the controlled fermentation of sourdough by *F. sanfranciscensis* with 10% sugar can be used as a biological improver and preservative to enhance the shelf-life and quality characteristics of bread in the baking industry, potentially replacing chemically acidified dough or the addition of propionic acid to produce a functional product.

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Conflict of interest

The authors declare that there is no conflict of interest.

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