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Use of natural yogurt, pineapple juice, and beer wort as starter cultures in sourdough combined with white and whole wheat flours to improve the technological characteristics of milk breads

Abstract

The objective of this study was to investigate the combined effects of different types of wheat flour (whole or white) with natural yogurt, pineapple juice, and beer wort for sourdough production to improve the technological characteristics of milk breads. Seven milk breads were prepared with varying yeast fermentation and sourdough types (produced with different types of wheat flour and substrates). A physical analysis (oven jump, bread shape, crust thickness, expansion coefficient, and images of the slices), proximal composition examination (moisture, carbohydrates, proteins, lipids, and ash), and acceptability and purchase intent evaluation were then performed. The results showed that the factors under study influenced the technological characteristics of the milk breads, and that the sourdough consisting of whole wheat flour and yogurt was less acceptable. However, sourdough prepared using white wheat flour and beer wort was the most effective, being a promising alternative in the elaboration of milk breads.

Keywords: fermentation, sourdough, baker's yeast, wheat flour

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Introduction

Bread is one of the most consumed foods globally[1] due to its low cost, pleasant taste, ease of preparation, high nutritional content, and source of energy, fiber, and protein.[2] Its preparation includes the use of ingredients such as wheat flour, water or milk, yeast, salts, and additives such as enzymes, hydrocolloids, and emulsifiers.[3,4] Recently, the increase in consumer demand for food products with high nutritional value and positive health effects has directed the bakery industry to produce more artisanal natural and sustainable products, including sourdough.[5,6] Sourdough is one of the oldest bioprocessing techniques, characterized by a mutual relationship between a complex microbiota dominated by yeast and lactic acid bacteria (LAB).[7] Although the use of commercial bakers' yeasts is quicker and simpler, the microbial diversity in sourdough has been shown to provide many additional benefits, including improved bread rheology, extended shelf life, enhanced nutritional attributes, and superior flavor.[8] During sourdough fermentation, microorganisms produce a variety of metabolites such as amino acids, short-chain fatty acids, and polypeptides.[9] Yeast produces CO_2, which improves the volume of the bread, while LAB promotes dough rheology and bread structure by producingorganic acids and extracellular polysaccharides and ads flavor by synthesizing volatile compounds.[10]

Sourdough can be classified into four types based on the production method used.[11] Sourdough type I has a long fermented time, as the microbiota of the flour, water, and the environment are responsible for fermentation.[12] For sourdough type II, the LAB are either solely, or in combination with yeast added as starter cultures, which ferment the dough for 15–24 h, and dehydration of this dough produces sourdough type III.[13] Type IV is a mixture of sourdough types I and II, in which culture starters or other inocula are added, such as fruit or honey, and the dough is fermented over a long duration.[14] Furthermore, spontaneous production depends on the microflora present in the flour.[15] Thus, the type of flour used also provides bread with different aromas and tastes.[16] To the best of our knowledge, the simultaneous effects of starter cultures made from yogurt, pineapple juice, and beer wort combined with white wheat and whole wheat flour on the technological characteristics of milk breads have not yet been studied. Therefore, this study aimed to investigate the combined effects of wheat flour types (white and whole) and the use of yogurt, pineapple juice, and beer wort for sourdough production to improve the technological characteristics of milk bread.

Materials and methods

This study was conducted at the Pilot Plant for Starchy Products and at the Sensory Analysis and Bromatology Laboratories of the School of Nutrition of the Federal University of Ouro Preto, Ouro Preto, Brazil. For the elaboration of the milk breads, beer wort, sugar free natural yogurt, pineapple juice *in natura*, whole wheat flour, white wheat flour, baker's yeast, sugar, milk, eggs, unsalted margarine, and salt were purchased from local shops.

Bread making

To evaluate the use of yogurt, pineapple juice, and beer wort as starter cultures in sourdough preparation combined with white and whole wheat flour, seven types of formulations were prepared using an experimental completely randomized design with three repetitions (Table 1). For the preparation of sourdough, the methodology proposed by Aplevicz et al.[17] was used with modifications. In summary, initially, each substrate was mixed with wheat flour (whole wheat flour or white wheat flour) and incubated for 24 h in a temperature-controlled chamber at 25 °C in the presence of oxygen in glass jars. Wheat flour (30 g, whole or white) and 20 g of substrate were added to the mixtures, which remained for another 24 h in a temperature-controlled chamber at 25 °C in the presence of oxygen. Then, 50 g of wheat flour (whole or white) and 30 g of filtered water were added to each mixture, which remained for another 24 h under the same conditions. Subsequently, 75 g wheat flour (whole or white) and 30 g filtered water were added to each mixture and incubated for 24 h in a temperature-controlled chamber at 25 °C in the presence of oxygen. Finally, 300 g of wheat flour (whole or white) and 200 g of filtered water were added to the yeasts every 24 h and stored under refrigeration (8 °C) per two days. The ready-made sourdoughs were stored in covered glass jars under refrigeration (8 °C), and every 15 days, the yeasts were fed in a 2:2:1 ratio (sourdough:wheat flour:water).

Table 1 Study design for preparation of milk bread formulations

Formulations	Ferment	White wheat flour (%)	Sugar (%)*	Milk (%)*	Egg (%)*	Margarine (%)*	Salt (%)*
CY	Baker's yeast (3%)	100	20	40	15	5	1
WY	White Wheat Flour + Natural Yogurt (25%)	100	20	40	15	5	1
IY	Whole Wheat Flour + Natural Yogurt (25%)	100	20	40	15	5	1
WP	White Wheat Flour + Pineapple Juice (25%)	100	20	40	15	5	1
IP	Whole Wheat Flour + Pineapple Juice (25%)	100	20	40	15	5	1
WW	White Wheat Flour + Beer Wort (25%)	100	20	40	15	5	1
IW	Whole Wheat Flour + Beer Wort (25%)	100	20	40	15	5	1

*In relation to the amount of white wheat flour.

As shown in Table 1, wheat flour, milk, sugar, salt, baker's yeast, and sourdough were mixed with a kneader (G Paniz, AE 05 L model) at a high speed for 7 min. Eggs and margarine were then added and mixed for another 5 min. After cutting and shaping, the dough was proofed in a climate chamber at 28 °C until it reached the maximum volume without loss of touch resistance, and then baked in an oven (Venâncio, Twister model) at 180 °C for 30 min. After baking, the bread was cooled to room temperature (25 ± 2 °C) for 1 h, packed in polypropylene bags previously sanitized with 70% alcohol and placed in a climate chamber at 25 °C and 75% humidity for later analysis.

Physical measurements

Oven jump was evaluated according to Shittu et al.[18] whereby the difference between the height of the dough at the end of fermentation and the height of the baked bread was determined using a caliper (mm). Bread shape was measured by dividing the height and width of the central portion of the bread.[19] Crust thickness (mm) was measured with a caliper at four different points, according to the methodology proposed by Pereira et al.[20] The expansion coefficient was determined by the method of Oliveira and Moraes[21], by dividing the difference between the volume of the raw dough and the volume of the baked bread by the volume of the baked bread and multiplying by 100. Three slices of each formulation were digitized, and the images were evaluated using ImageJ software to quantify the number of alveoli per cm^2, percentage of air, average size of the alveoli (mm^2), circularity (mm), perimeter of the alveoli (mm), and total area (mm^2), according to the methodology proposed by Oliveira et al.[22]

Proximal composition

Moisture, ash, protein content (micro-Kjeldahl method), and lipid content (Soxhlet method) of the final product were analyzed following the methods of AOAC[23]. The carbohydrate content was determined using the Antrona method.[24]

Sensory analysis

The sensory evaluation of bread was conducted using an acceptance test with 80 consumers (aged 18–50 years). The analysis was conducted in individual air-conditioned (22 °C) booths under white light. Water was provided for palatal cleansing. Sessions were held at the Laboratory of Sensory Analysis (Federal University of Ouro Preto), and samples (a quarter of a slice of bread including dough and crust) were presented on white disposable plates with three-digit numbers randomly coded.[25] Approval for the study was obtained from the Ethics Committee of the Federal University of Ouro Preto (Number 4144363), and written consent was obtained from all volunteers. Consumers evaluated appearance, aroma, taste, and texture using a structured nine-point hedonic scale (1-dislike extremely, 2- dislike very much, 3-dislike, 4-dislike slightly, 5-neither like nor dislike, 6-like slightly, 7-like, 8-like very much, and 9-like extremely). In addition, the intention to buy was evaluated using the attitude scale (1 = certainly would not buy to 5 = would certainly buy).[26]

Statistical analysis

Analysis of variance (ANOVA) and the Scott-Knott test were performed for all results using the statistical program Sisvar[27] at a confidence interval of 95%. For easy viewing of the sensory acceptance of the bread formulations and to correlate it with the physical parameters, a three-way external preference map obtained by PARAFAC was used.[28] A three-way array was arranged from matrices of i rows (i samples) and j + m columns (j consumers + m physical measurements).[29] The PARAFAC model was optimized using the value of core consistency diagnostics (CORCONDIA) to select the number of factors.[28–30]

Results and discussion

Table 2 shows the average results of the physical measurements of the different milk bread formulations. The oven jump occurs during the heating of the dough in the oven, causing the expansion of gases and the vaporization of water[31] and heat is transported by conduction inside the bread according to Fourier's law. Effective thermal conduction is used to incorporate the evaporation–condensation mechanism in the heat transfer[32] with a rapid increase in bread volume that ceases after starch gelatinization.[33] There were no differences between the prepared breads in relation to the oven jump ($p>0.05$), indicating that the use of different ferments did not change the increase in bread volume during baking; that is, the ferments in the present study did not cause distinct phase changes in milk bread.[34,35]

According to Bodroža-Solarov et al.[19] loaf shape values greater than 0.5 indicate spherical shapes. Therefore, all the breads prepared had a spherical shape (Table 2), while the breads made with baker's yeast (CY) and whole wheat flour and yogurt (IY) had higher mean values ($p \leq 0.05$) but did not differ from each other. In general, the visual appearance and shape of food are important factors in eating behavior.[36] The milk breads made with baker's yeast (CY), white wheat flour, beer wort (WW), and with whole wheat flour and yogurt (IY) had lower crust thickness values ($p \leq 0.05$) but did not differ from each other (Table 2). Crust thickness is directly linked to moisture, and as the moisture increases the crust becomes thicker.[37] The crust

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is formed as the moisture is released from the inside of the bread to the outside during heating in the oven; therefore, as more moisture is released, the crust increases in thickness.[38] In the study by Hayta and Ertop[39] crust thickness values were found of 4.29 mm, 4.27 mm, and 3.46 mm, and moisture of 39.68%, 38.20%, and 38.11%, respectively, for breads made with sourdough inoculated with LAB, uncontrolled sourdough, and baker's yeast, indicating that moisture is a determining factor for the crust thickness. In the present study, proportionality was observed between the moisture content Table 4 and crust thickness (Table 2).

Table 2 Mean values of the physical measurements of the different milk bread formulations

Formulations	Oven jump (mm)	Bread shape	Crust thickness (mm)	Expansion coefficient (%)
CY	10.6±1.68 a	1.36±0.14 a	0.74±0.42 b	255.67±19.63 a
WY	9.94±0.42 a	0.62±0.08 c	1.21±0.26 a	126.67±23.09 c
IY	7.01±1.32 a	1.26±0.12 a	0.88±0.20 b	167.00±0.00 b
WP	13.81±2.13 a	0.86±0.03 b	1.41±0.40 a	167.00±0.00 b
IP	10.42±2.05 a	0.69±0.05 c	1.40±0.29 a	144.33±19.63 c
WW	10.14±2.63 a	0.68±0.02 c	1.02±0.18 b	126.67±23.09 c
IW	10.62±3.24 a	0.79±0.08 b	1.60±0.61 a	133.00±0.00 c

Note: n=3. Means±standard deviation followed by the same letter in the column do not differ from each other by the Scott-Knott test at 5% probability. baker's yeast (CY); sourdough: white wheat flour and yogurt (WY), whole wheat flour and yogurt (IY), white wheat flour and pineapple (WP), whole wheat flour and pineapple (IP), white wheat flour and must of beer (WW), whole wheat flour and beer wort (IW).

The breads made with baker's yeast (CY) had the highest mean expansion coefficient ($p \leq 0.05$) (Table 2). According to Oliveira and Moraes[21] breads with higher expansion coefficients are more aerated and lighter in weight. As it contains only yeasts potentiated for CO_2 production, baker's yeast (CY) provides better results than sourdough, thus expanding from sourdough, which is composed of yeasts and LAB that produce less CO_2.[40] The physical characteristics of the slices of bread made with sourdough and baker's yeast are shown in Table 3. The quality of the bread is determined by the aerated crumb, with a uniform and symmetrical distribution of the alveoli.[41] According to Tasiguano et al.[42] higher mean values for alveolar count and percentage of air, and lower values for the mean size of the alveoli demonstrate a greater ability of the crumb to retain air, thus making it soft and voluminous. According to the same authors, a low number of alveoli symbolizes a hard and small bread. In addition, data on perimeter and circularity are correlated with regularity and symmetry, as high alveolar perimeters symbolize greater irregularity in the same area and circularity is an indicator of the shape of the alveoli (between 0 and 1).[41] According to Scheuer et al.[43] a circularity close to zero (0) corresponds to more elongated alveoli, while that close to one (1) relates to round alveoli. The breads made with baker's yeast (CY) and IY (whole wheat flour and yogurt) had higher amounts of alveoli per cm^2. Bread made with white wheat flour and beer wort (WW) had a higher percentage of air. Thus, it can be inferred that the CY, IY, and WW breads were soft, which may be related to the ability to produce and retain CO_2, since the alveoli are formed when the carbon dioxide is trapped in the dough, making the bread develop its volume and provide softness.[42]

Breads made with only white wheat flour (CY, WY, WP, and WW) had more circular alveoli than breads made with whole-meal flour. (Table 3) Zambelli et al.[44] reported that circularity is influenced by the incorporation of ingredients, some of which may hinder gluten network development. As whole wheat flour is low in gluten, its incorporation into bread makes it difficult for the gluten network to develop because 25% of sourdough is incorporated into the dough.[45] Regarding the perimeter of the alveoli, it was observed that breads made with WY (white wheat flour and yogurt) and IP (whole wheat flour and pineapple) sourdough had higher mean values and did not differ from each other (Table 3). Correa et al.[46] reported that larger perimeters are associated with lower alveolar regularity in the same area. Breads made with IP sourdough (whole wheat flour and pineapple) had a greater total area ($p \leq 0.05$) (Table 3). The alveolar area is related to the lamellar viscosity of gluten; that is, higher values results in lower viscosities, which helps the expansion of the alveoli.[46] The breads produced with CY (baker's yeast) and IY (whole wheat flour and yogurt) had lower moisture and higher carbohydrate values (Table 4) ($p \leq 0.05$). Moisture results are inversely proportional to carbohydrate results since liquids solubilize carbohydrates.[47] Table 3 shows that these yeasts have larger slice areas, indicating greater water evaporation.[48]

The breads made with the CY (baker's yeast), WY (white wheat flour and yogurt), WP (white wheat flour and pineapple), IP (whole wheat flour and pineapple), and IW (whole wheat flour and beer wort) showed higher mean protein values ($p \leq 0.05$) (Table 4). Some yeasts, such as *Kluyveromyces lactis, Saccharomyces cerevisiae*, and *Candida utilis*, can synthesize protein during growth, which significantly increases the protein content. These organisms also release nitrogen compounds during fermentation, which influences the protein value.[49] Breads made with WP (white wheat flour and pineapple) and IP (whole wheat flour and pineapple) sourdoughs had higher lipid values ($p > 0.05$) (Table 4). According to Gänzle et al.[50] certain bacteria are able to produce lipids, as in the case of *Lactobacillus sanfranciscensis*, which may be abundant in pineapple. In addition, pineapple contains *Leuconostoc citreum*, which is a leuconostoc bacterium that has a strong interaction with the lipase enzyme present in wheat flour, thus breaking the lipids in the dough and making it available to final product.[51] This process is promoted by the starch-lipid complexation, which prevents the action of enzymes in other sourdoughs.[51] Milk breads made with CY (baker's yeast) and IW (whole wheat flour and beer wort) had higher and lower mean ash values, respectively ($p \leq 0.05$) (Table 4). Ash is inversely proportional to liquid absorption.[52]

The mean sensory scores obtained from the consumer acceptance tests are presented in Table 5. Through ANOVA, a significant difference was found between the milk breads prepared for all sensory parameters evaluated ($p \leq 0.05$). In relation to the characteristics of appearance Table 5 aroma, taste, and texture, all treatments received an average score between 6.35 and 8.05, ranging from "I liked it slightly" to "I liked it very much," indicating that the tasters accepted the elaborate milk breads. However, in relation to purchase intention, it was observed that the scores ranged from 2.91 to 3.89 ("probably would not buy" to "probably would buy").

In general, breads made with IY (whole wheat flour and yogurt) sourdough showed lower acceptance for all sensory attributes and lower purchase intent Table 5. This result may be due to the whole wheat flour used in the preparation of sourdough, which provided the appearance of whole-grain breads, promoting browning of the dough, and consequently, providing less uniformity to the crumb.[53,54] A three-way external preference map was generated Figure 1 by analyzing the parallel factors (PARAFAC) to represent the distribution of the 80 consumers (vectors), the milk bread formulations (CY, WY, IY, WP, IP, WW, and IW), and acceptance data in relation to sensory attributes (appearance, aroma, flavor, texture, and purchase intent), and to correlate preferences with the data from the physical analysis. PARAFAC was fixed with a two-factor model, which explained 36.48% of the variability and presented a CORCONDIA value of 79.45%.

Table 3 Mean values of the physical characteristics of the slices of bread made with sourdough and baker's yeast

Formulations	Number of alveoli per cm²	Air percentage	Mean alveoli size (mm²)
CY	745.33±48.19a	43.44±1.88d	0.67±0.04d
WY	658.33±28.38b	41.21±0.47e	0.56±0.01d
IY	726.67±3.79 a	47.58±0.44 c	5.94±0.09b
WP	379.67±8.50d	36.78±1.38 f	0.77±0.05d
IP	495.33±17.62c	48.34±0.35 c	8.88±0.37a
WW	327.33±7.77e	67.87±0.04a	2.55±0.01c
IW	163.33±16.29f	50.53±2.88 b	2.84±0.42c
Formulations	**Circularity (mm)**	**Perimeter of the alveoli (mm)**	**Total area (mm²)**
CY	0.77±0.01c	0.85±0.00c	501.54±27.03d
WY	0.81±0.00a	0.87±0.00a	374.42±12.23e
IY	0.24±0.00f	0.85±0.00c	4318.81±39.77b
WP	0.74±0.01d	0.83±0.00d	291.03±11.09f
IP	0.24±0.00f	0.87±0.00a	4395.32±40.23a
WW	0.80±0.00b	0.86±0.00b	835.45±22.23c
IW	0.73±0.00e	0.85±0.00c	458.7±26.12d

Note: n=3. Means±standard deviation followed by the same letter in the column do not differ from each other by the Scott-Knott test at 5% probability. baker's yeast (CY); sourdough: white wheat flour and yogurt (WY), whole wheat flour and yogurt (IY), white wheat flour and pineapple (WP), whole wheat flour and pineapple (IP), white wheat flour and must of beer (WW), whole wheat flour and beer wort (IW).

Table 4 Mean values of the proximal composition of milk breads made with sourdough and baker's yeast

Formulations	Moisture (%)	Carbohydrate (%)	Protein (%)	Lipid (%)	Ash (%)
CY	26.00±0.00 c	61.38±0.93 a	8.57±0.79 a	2.40±0.14 b	1.65±0.01 a
WY	29.67±1.15 b	57.49±1.48 c	8.99±0.53 a	2.38±0.17 b	1.48±0.01 d
IY	26.33±2.52 c	61.95±2.36 a	7.16±1.40 b	2.03±0.18 b	1.53±0.01 c
WP	30.00±1.00 b	56.25±1.22 c	9.01±0.33 a	3.19±0.20 a	1.55±0.03 c
IP	31.67±0.58 a	54.45±0.55 d	9.15±0.58 a	3.15±0.22 a	1.58±0.02 b
WW	29.33±0.58 b	59.11±0.42 b	7.67±0.21 b	2.36±0.37 b	1.53±0.01 c
IW	32.67±0.58 a	55.09±0.05 d	8.51±0.71 a	2.32±0.17 b	1.41±0.01 e

Note: n=3. Means±standard deviation followed by the same letter in the column do not differ from each other by the Scott-Knott test at 5% probability. baker's yeast (CY); sourdough: white wheat flour and yogurt (WY), whole wheat flour and yogurt (IY), white wheat flour and pineapple (WP), whole wheat flour and pineapple (IP), white wheat flour and must of beer (WW), whole wheat flour and beer wort (IW).

Table 5 Mean values of sensory attributes and purchase intention of milk breads made with sourdough and baker's yeast

Formulations	Appearance	Aroma	Taste	Texture	Purchase intention
CY	7.59±1.40 b	7.31±1.61 a	7.42±1.63 a	7.38±1.62 b	3.71±1.16 a
WY	7.56±1.47 b	7.36±1.37 a	7.48±1.63 a	7.33±1.61 b	3.75±1.08 a
IY	6.48±1.97 d	6.35±2.00 b	6.39±2.01 b	6.44±1.88 c	2.91±1.28 b
WP	7.83±0.95 a	7.33±1.1 3a	7.45±1.43 a	7.32±1.21 b	3.83±1.11 a
IP	7.01±1.54 c	7.38±1.02 a	7.25±1.36 a	7.34±1.19 b	3.70±1.08 a
WW	8.05±0.94 a	7.78±0.93 a	7.95±0.90 a	7.78±1.01 a	4.13±1.15 a
IW	7.55±1.14 b	7.45±0.97 a	7.48±1.16 a	7.61±1.00 a	3.89±0.98 a

Note: n=80. Means±standard deviation followed by the same letter in the column do not differ from each other by the Scott-Knott test at 5% probability. baker's yeast (CY); sourdough: white wheat flour and yogurt (WY), whole wheat flour and yogurt (IY), white wheat flour and pineapple (WP), whole wheat flour and pineapple (IP), white wheat flour and must of beer (WW), whole wheat flour and beer wort (IW).

Breads made with WW (white wheat flour and beer wort), WP (white wheat flour and pineapple), and IW (whole wheat flour and beer wort) yeasts were more accepted. As shown in Table 5, these formulations presented scores varying between the hedonic phrases "I liked it moderately" and "I liked it extremely" with purchase intention between "I don't know if I would buy it" and "I would certainly buy it." These breads showed the same oven jump (Table 2), but the breads made with WP and IW sourdoughs were characterized by a greater crust thickness than those of WW breads, indicating that WP and IW breads had higher moisture content than that of WW. Breads made with IY (whole wheat flour and yogurt) sourdough showed lower acceptance, with scores ranging from "liked slightly" to "liked moderately" with purchase intention between "probably would not buy" and "I don't know if I would buy it." This suggests that the use of whole wheat flour contributed negatively to the acceptance of breads made with the yogurt substrate, since the use of white wheat flour with the same substrate showed an increase in acceptance shown in Figure 1.

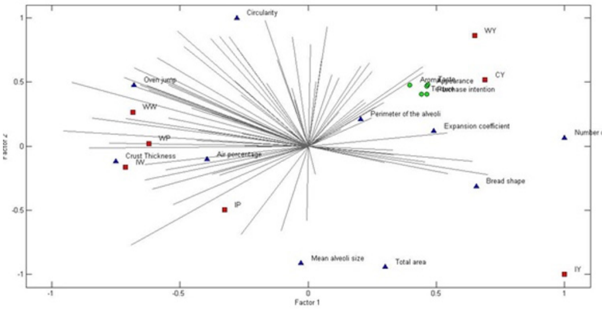

Figure 1 Three-way external preference map for sensory attributes and physical properties of milk breads.

Conclusion

The use of yogurt, pineapple juice, and beer wort as starter cultures in sourdough combined with white and whole wheat flours significantly influenced the technological characteristics of the prepared breads, with the exception of oven jumps. Although the use of some sourdoughs provided greater crumb irregularity (WY and IP) and increased lipid levels (WP and IP), this is a promising approach to improve the technological and nutritional properties of milk bread. Sourdough fermentation promoted modifications compared to that of baker's yeast, mainly in relation to cell expansion and acceptability. Breads made with baker's yeast (CY) and IY (whole wheat flour and yogurt) and WW (white wheat flour and beer wort) sourdoughs had lower crust thickness and greater softness, which are important factors in the choice of products. However, IY bread showed lower acceptance, probably because of the use of whole wheat flour, which provided the appearance of whole-grain breads, promoting browning of the dough. Overall, among the sourdoughs used in this study, sourdough prepared using white wheat flour and beer wort was the most effective, making it a promising alternative for milk breads.

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Conflicts of interest

The author declares no conflicts of interest.

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