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Isolation and identification of lactic acid bacteria with proteolytic potential from *sourdough* produced in Londrina, Paraná, Brazil

Isolamento e identificação de bactérias láticas com potencial proteolítico a partir de *sourdough* produzidas em Londrina, Paraná, Brasil

Roger Henrique Cano[1]; Karla Bigetti Guergoletto[2]*; Sandra Garcia[2]

Highlights

Investigate LAB that hydrolyze wheat proteins to reduce allergens.
Seven LAB strains were isolated from sourdoughs produced in Londrina.
Companilactobacillus crustorum and *Lactiplantibacillus plantarum* were identified.

Abstract

Wheat flour is one of the most common causes of food allergies, mainly because of the presence of gluten. The growing number of individuals with some sensitivity or pathology related to wheat proteins and scarcity of therapies for treatment (as simply adopting a gluten-free diet can be complex) demonstrate the need for alternative treatments. The present study aimed to isolate and identify lactic acid bacteria (LAB) from long-fermented bread dough (sourdough), with the potential to hydrolyze allergenic wheat proteins. Three different dough samples were collected from sourdough production establishments in Londrina, PR, USA. The breads were prepared in a laboratory. As positive controls, two formulations were prepared using only biological yeast containing lyophilized *Saccharomyces cerevisiae.* The fermentation process was monitored based using pH and acidity values, and the amount of gluten after fermentation was determined using the gravimetric method. LAB isolated from sourdough were inoculated into a medium containing gluten as the sole nitrogen source to evaluate the hydrolysis potential of wheat protein. Microorganisms were identified using biochemical and genotypic tests, genomic amplification using polymerase chain reaction and subsequent sequencing of the 16S region of ribosomal RNA. Seven LAB isolates were identified, including *Lactiplantibacillus plantarum* and *Companilactobacillus crustorum.* Compared with traditional fermentation, sourdough shows significant changes in pH and acidity during fermentation. There was a significant reduction in dry gluten (19.90%) (w/w) at the end of sourdough fermentation compared with that of traditional dough (*S. cerevisiae*). Although sourdough

[1] Master's Degree, Graduate Program in Food Science. Departament of Food Science and Technology, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil. E-mail: roger.h.cano@gmail.com
[2] Prof[as] Ph.D., Graduate Program in Food Science, Departament of Food Science and Technology, UEL, Londrina, PR, Brazil. E-mail: karla@uel.br; sgarcia@uel.br
* Author for correspondence

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does not completely eliminate gluten, it can reduce the amount of gluten and improve the digestibility of wheat-based products. Further studies could be intersting to assess their impact on allergenicity in individuals sensitive to wheat proteins.
Key words: Natural fermentation. Gluten. PCR. *Companilactobacillus crustorum. Lactiplantibacillus plantarum.*

Resumo

A farinha de trigo é uma das causadoras mais comuns de alergia alimentar e isto se deve principalmente à presença do glúten. O crescente número de indivíduos portadores de alguma sensibilidade às proteínas do trigo, aliado à escassez de terapias para tratamento, já que a simples adoção de uma dieta isenta de glúten pode ser complexa, demonstra a necessidade de que alternativas sejam buscadas. O presente estudo teve por objetivo isolar e identificar bactérias láticas (BAL) em massas de pães de fermentação natural prolongada (*sourdough*), com potencial de hidrolisar as proteínas alergênicas do trigo. Três massas diferentes foram coletadas em estabelecimentos produtores de *sourdough* na região de Londrina – PR, Brasil. Em seguida, os pães foram reproduzidos em laboratório. Como controle positivo, foram produzidas duas formulações utilizando apenas fermento biológico comercial liofilizado contendo *Saccharomyces cerevisiae.* O processo fermentativo foi acompanhado por medidas de pH e acidez, e a quantidade de glúten após as fermentações foi determinada pelo método gravimétrico. As BAL isoladas a partir das *sourdough* foram inoculadas em meio contendo glúten como única fonte de nitrogênio, para avaliação do potencial de hidrólise da proteína do trigo. A identificação dos microrganismos foi realizada por testes bioquímicos e genotípicos por amplificação genômica através da técnica de Reação em Cadeia da Polimerase (PCR) e posterior sequenciamento da região 16S do DNA ribossomal. No total, foram obtidos sete isolados de BAL que foram identificados como *Lactiplantibacillus plantarum* e *Companilactobacillus crustorum.* Em comparação a fermentação tradicional, as *sourdough* apresentaram alteração significativa de pH e acidez durante a fermentação, além de redução significativa de 19,90% (m/m) de glúten seco quando comparados às massas tradicionais (*S. cerevisiae*). Embora o sourdough não elimine completamente o glúten, ele pode reduzir sua quantidade e melhorar a digestibilidade de produtos à base de trigo. No entanto, são necessários estudos adicionais para avaliar seu impacto na alergenicidade em indivíduos com sensibilidade às proteínas do trigo.
Palavras-chave: Fermentação natural. Glúten. PCR. *Companilactobacillus crustorum. Lactiplantibacillus plantarum.*

Introduction

Wheat flour is a raw material widely used in many cultures for food production, particularly bread. However, the allergenic potential of gluten-forming wheat proteins affects an increasing number of individuals with celiac disease, irritable bowel syndrome, and other allergies and sensitivities. Consequently, efforts have been made to mitigate this issue (Papadimitriou, 2019; Gobbetti et al., 2019).

The reduction of gluten content in baked goods has been studied using lactic acid bacteria (LAB), which are capable of hydrolyzing proteins during the fermentation of wheat-based products. Therefore, it is important to evaluate the proteolytic strains capable of reducing the allergenic potential of wheat flour (Prandi et al., 2017; Nionelli & Rizzello, 2016; Papadimitriou, 2019).

Sourdough is a naturally fermented dough used worldwide to produce a variety of baked goods. From a microbiological perspective, it can be considered a specific ecosystem hosting yeast and LAB (Oshiro et al., 2021).

The main difference between sourdough and dough produced with commercial baker's yeast and short fermentation times is the contribution of the microbial community to metabolic conversion within the dough (Menezes et al., 2021).

According to Papadimitriou (2019), sourdoughs can be classified into four types: Type I, involving spontaneous fermentation at room temperature followed by back-slopping to maintain the process; Type II, involving fermentation at higher temperatures (≥30°C) and the use of semi-fluid starters to acidify the dough; Type III, similar to Type II but pasteurized or processed for extended shelf life; and Type IV, a combination of Types I and II.

Prolonged natural fermentation can improve the quality of baked products, adding health benefits, such as reduced glycemic response (Takemura et al., 2024), lower acrylamide levels, enhanced sensory quality (Ameur et al., 2024), and increased shelf life (Arámburo-Gálvez et al., 2020; Nionelli & Rizzello, 2016).

In stabilized sourdoughs, the dominant LAB communities belong to the *Lactobacilli* genus, with heterofermentative species being the most frequently isolated (Gobbetti et al., 2016). *Lactobacillus sanfranciscensis, Lactobacillus fermentum,* and *Lactobacillus paralimentarius* recently reclassified by Zheng et al. (2020) as *Fructilactobacillus sanfranciscensis, Limosilactobacillus fermentum,* and *Companilactobacillus paralimentarius,* respectively (primarily isolated from sourdough niches) along with *Lactobacillus plantarum* and *Lactobacillus brevisnow* classified as *Lactiplantibacillus plantarum* and *Levilactobacillus brevis*, which are commonly found in a variety of fermented foods are among the most abundant species in sourdoughs (Corsetti et al., 2007; Martino et al., 2016; Zheng et al., 2015, 2020).

Thus, the proteolytic properties of LAB, combined with the sensory benefits developed during fermentation, present a promising avenue for the development of processes and wheat flour-based products with reduced allergenic potential. The aim of the present study is to isolate and identify potentially proteolytic LAB strains from naturally fermented sourdoughs produced in the Londrina region of Paraná, Brazil, and evaluate the pH, acidity, and gluten content of these doughs.

Materials and Methods

Sample collection

Three samples of naturally fermented bread dough were collected from three different establishments in the city of Londrina, Paraná, Brazil (-23.29742, -51.20427). Each sample weighed 100 g, representing at least 20% of the volume of the starter dough (dough used as the inoculum for fermentation).

The samples were labeled S1, S2, and S3, and traditionally produced without the use of chemicals or commercial yeast, as shown in Table 1. The doughs obtained were classified as Type I sourdoughs, which involve the daily propagation and maintenance of microorganisms in a metabolically active state.

Table 1
Fermentation conditions and characteristics of the collected dough samples

Parameter	S1	S2	S3
Storage temperature	4ºC	6ºC to 7ºC	8ºC to 16ºC
Physical state	Solid	Solid	Solid
Starter origin	Water, wheat flour, and apricot (approx. 30 days)	Water, wheat flour, and pineapple juice (approx. 30 days)	Water and wheat flour (approx. 30 days)
Cultivation time	7 years	5 years	8 years

The collected doughs were stored at 6°C in sterilized, airtight containers until the beginning of the experiment, which occured 3 h after collection.

Dough preparation and fermentation

Five dough samples were prepared for fermentation. In experiments 1, 2, and 3, the collected sourdough samples (S1, S2, and S3) were used as the fermenting agent, whereas in experiments 4 and 5, dry commercial yeast containing only *Saccharomyces cerevisiae* (Fleischmann, Petrópolis, Brazil), purchased from a local market, was used.

The sourdough-based doughs were prepared using wheat flour (50% w/w), sourdough from the previous fermentation (15% w/w), water (33.8% w/w), and salt (1.2% w/w), and mixed for 10 min. In the dough made with commercial yeast, the sourdough was replaced with lyophilized yeast according to the manufacturer's instructions.

After mixing, the doughs from experiments 1, 2, 3, and 4 were stored at 21°C for 48 h. Every 12 h, pH and titratable acidity were measured. At the end of the 48-h fermentation, 50 g of each dough was collected for gluten extraction and quantification. In experiment 5, the dough

was stored 25ºC for 2 h, following the manufacturer's recommendations for commercial yeast, to accurately simulate the current bread-making process.

pH, acidity, and gluten quantification

The pH and titratable acidity of the fermented dough were determined according to the American Association of Cereal Chemistry [AACC] (2010).

Gluten content was measured in the prepared dough, as described above, using the gravimetric method (Instituto Adolfo Lutz [IAL], 1985). A 50-g portion of each sample was weighed and 10 mL of a 5% aqueous sodium chloride solution was added. The mixture was stirred using a glass rod until a compact agglomerated dough was formed, which was then left to rest for 30 min. Water was then added to fully cover the dough, followed by a 30-min resting period.

The dough was then washed under running water over a 100-mesh sieve, gently pressed, and kneaded by hand. This process continued until the rinse water no longer turned blue upon the addition of a drop of saturated iodine solution, indicating complete starch removal.

The remaining sample was transferred to a watch glass that had been pre-heated in an oven at 105°C for 1 h and cooled in a desiccator to room temperature before weighing. The watch glass with the dough was then returned to an oven at 105°C for 5 h. Subsequently, the samples were cooled to room temperature in a desiccator and weighed. Heating and cooling steps were repeated until a constant weight was attained.

The amount of dry gluten obtained from the naturally fermented doughs was compared to the amount (% w/w) of gluten obtained from short-fermentation doughs traditionally leavened with yeast (*S. cerevisiae*). The percentage of dry gluten was calculated as the ratio of dry gluten (g) to sample mass (g).

Isolation and identification of proteolytic LAB

Samples S1, S2, and S3 (25 g each) were cultivated in 225 mL of Man-Rogosa-Sharpe (MRS) broth (Difco, Franklin Lakes, NJ, USA) at 37°C for 48 h. Next, 25 mL of each initial inoculum (in MRS broth) was centrifuged (8,000 xg) (Eppendorf 5810R - Eppendorf do Brasil Ltda, Sumarezinho – SP, Brazil), for 5 min and washed with saline solution (0.85%, v/v). The pellets were subsequently transferred to tubes containing 225 mL of gluten base medium, composed of wheat flour gluten (9%), glucose (2%), KH_2PO_4 (1%), K_2HPO_4 (1%), Tween-80 (0.1%, v/v), and distilled water, and incubated at 37°C for 24 h (Gerez et al., 2006).

For microorganism isolation, a serial dilution (9:1) was performed using saline solution (0.85%), and dilutions from 10^{-4} to 10^{-8} were plated, in duplicate, on MRS agar using the surface spread technique, and incubated at 37°C for 48 h (Gerez et al., 2006). Colonies with distinct morphologies were streaked onto MRS agar for phenotypic and genotypic identification.

Phenotypic identification was performed according to the method proposed by Carr et al. (2002). Gram staining was used to assess the cell morphology and Gram reaction, followed by catalase testing and gas

production from glucose fermentation. Based on these results, an additional confirmatory test growth at 15°C was conducted to determine the group of the isolates.

For genotypic analysis, the isolates were sequenced for species-level identification. DNA was extracted using the PuriLink Genomic DNA Mini Kit (Invitrogen, São Paulo, Brazil), following the manufacturer's instructions, and quantified using a NanoDropLite spectrophotometer. Genetic material was subjected to PCR targeting the *16S* ribosomal RNA (rRNA) region using the following primers: forward CCGAATTCGT CGACAACAGAGTTTGATCCTGGCTCAG and GGATCCAAGCTTAAGGAGGTGATCCAGCC. This rRNA region is commonly used to identify LAB because it is highly conserved among these species (Clarridge, 2004). After amplification, the samples were purified using the PureLink PCR Purification Kit (Invitrogen) and sequenced at the Animal Virology Laboratory of the State University of Londrina using an AB13500 sequencer platform (Applied Biosystems, Foster City, CA, USA). The sense and antisense sequences were analyzed and aligned using the BioEdit software and then submitted to the BLASTn database of the National Center for Biotechnology Information to confirm the molecular identity of the samples.

Evaluation of acidification and proteolysis capacity of isolated LAB

For comparative analysis of the acidification effect of the doughs caused by the growth of LAB with proteolytic activity, two sourdoughs containing the species isolated from the samples were activated: one containing only *L. plantarum* and the other containing only *C. crustorum.* Fermentation was initiated by inoculation with these microbial cultures and propagated by daily backslopping, as shown in Figure 1.

For this, the isolated LAB were transferred to 10 mL of MRS broth for 48 h at 37°C, followed by centrifugation and suspension in 10 mL of saline solution. This solution was added to the dough, which contained 50 g of wheat flour (Venturelli, Sertanópolis, Brazil) and 50 g of water (1:1), and stored at 24°C for 48 h, completing step 1. In step 2, 25 g of the fermented dough was taken and inoculated into 50 g of wheat flour (Venturelli) and 50 g of water (1:2:2), and stored for another 48 h at 24°C. From the third step onwards, the time for the next inoculation was reduced to 24 h, and after the fourth step, the storage temperature was reduced to 21°C. The same mass ratio (1:2:2) was maintained until the final step (Menezes et al., 2021).

Thus, four new dough samples were obtained: one with a starter containing *L. plantarum,* two with a starter containing *C. crustorum;* and 3 and 4 with a starter containing *S. cerevisiae.* The doughs were prepared with 50% (w/w) wheat flour, 15% (w/w) starter dough, 33.8% (w/w) water, and 1.2% (w/w) salt, and mixed for 10 min. Doughs 1, 2, and 3 were stored at 21°C for 48 h. Every 12 h, pH and titratable acidity were evaluated. In experiment 4, the dough were stored at room temperature for 2 h to follow the recommendations of the biological yeast manufacturer, thus simulating the bread-making process used today.

At the end of the fermentation period, 50 g of each dough was collected for gluten extraction and quantification, as described above.

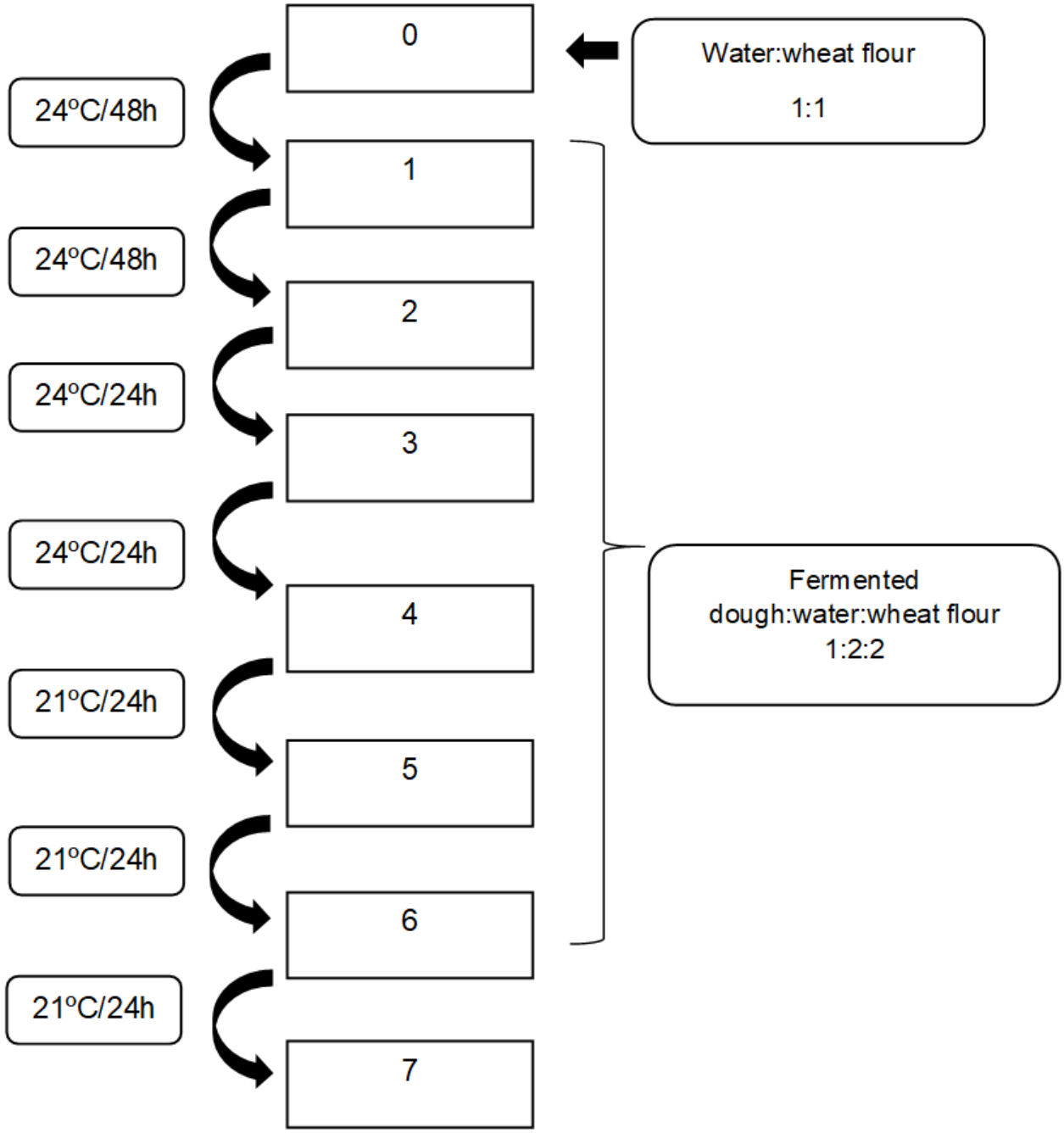

Figure 1. Production of fermented doughs with the addition of isolated LAB.

Statistical analysis

The analyses were performed in triplicate and the results are expressed as mean ± standard deviation. Tukey's test was used and the significance was set as p≤0.05. The data were analyzed using the Statistica 8.0 program (Statsoft Inc, 2007).

Results and Discussion

Determination of pH, acidity, and gluten in fermented doughs

The variations in pH and acidity during fermentation are shown in Table 2. Both parameters changed significantly (p<0.05) in the sourdough samples over the 48-h fermentation period. The pH decreased by 19.7% in S1, 14.51% in S2, and 21.30% in S3. The pH and titratable acidity values of doughs prepared with *S. cerevisiae* changed only slightly during 48 h of fermentation, with a pH reduction of 5.15%. Compared with baker's yeast, sourdoughs showed a four-fold increase in acid concentration, demonstrating greater acidification capacity than that of traditional dough. Increased lactic acid concentration in wheat-based fermented dough enhances the solubility of glutenins (Thiele et al., 2004). According to Nionelli and Rizzello (2016), the presence of LAB and their metabolic activity during fermentation are crucial for the activation of endogenous enzymes in flour. This leads to gluten depolymerization, mainly through the weakening of glutenin, aiding in the reduction of allergenic compounds (W. Fu et al., 2023; Menezes et al., 2021).

Table 1
Mean and standard deviation of pH and titratable acidity (TA) during the fermentation of sourdough doughs and with yeast (Saccharomyces cerevisiae) over time (T)

T	S1		S2		S3		4	
	pH	TA	pH	TA	pH	TA	pH	TA
0	5,43^{a} ± 0,03	8,30^{a} ± 0,57	5,03^{a} ± 0,02	9,60^{a} ± 0,57	5,07^{a} ± 0,05	10,00^{a} ± 2,00	5,24^{a} ± 0,02	2,30^{a} ± 0,57
1 (12h)	4,704^{b} ± 0,02	11,00^{b} ± 1,00	4,66^{b} ± 0,01	12,30^{b} ± 1,52	4,41^{b} ± 0,04	12,30^{a} ± 2,08	5,12^{b} ± 0,02	3,30^{a} ± 1,52
2 (24h)	4,43^{c} ± 0,02	16,60^{c} ± 2,82	4,31^{c} ± 0,02	18,00^{c} ± 1,00	4,25^{c} ± 0,02	18,60^{b} ± 0,57	5,00^{c} ± 0,03	3,00^{a} ± 1,00
3 (48h)	4,36^{d} ± 0,02	17,60^{c} ± 0,57	4,30^{c} ± 0,04	17,60^{c} ± 2,51	3,99^{d} ± 0,02	20,30^{b} ± 1,52	4,97^{c} ± 0,05	5,60^{b} ± 1,15

Mean values in the same column, representing the same evaluated parameter, followed by different letters are significantly different (p≤0.05). Mean values ± SD of triplicate determinations.
Legend: S1 – Water, wheat flour, and apricot; S2 - Water, wheat flour, and pineapple juice; S3 - Water and wheat flour; 4 - *Saccharomyces cerevisiae* for 48h.

After fermentation, the samples were subjected to gluten extraction (Figure 2). The naturally fermented samples showed a significant reduction ($p<0.05$) in dry gluten content compared with that of dough fermented with yeast for a short period (2 h). According to Nionelli and Rizzello (2016), the fermentation time is important for biochemical reactions and protein degradation. Additionally, microbial acidification shifts the pH of dough to values between 3.5 and 4.5, which coincides with the optimal pH range for cereal aspartic proteinases, key enzymes in primary proteolysis, and the specific proteolytic activity of LAB strains, supporting the findings of this study. A pH decrease in the dough promotes a greater reduction in gluten disulfide bonds, increasing solubility, and making it more susceptible to proteolytic degradation (Gobbetti et al., 2019; Thiele et al., 2004). During yeast fermentation, no marked acidification of the dough was observed, and the pH did not reach ideal values for primary proteolysis. This may favor greater integrity of the gluten structure, as reflected by a higher percentage of dry gluten at the end of fermentation.

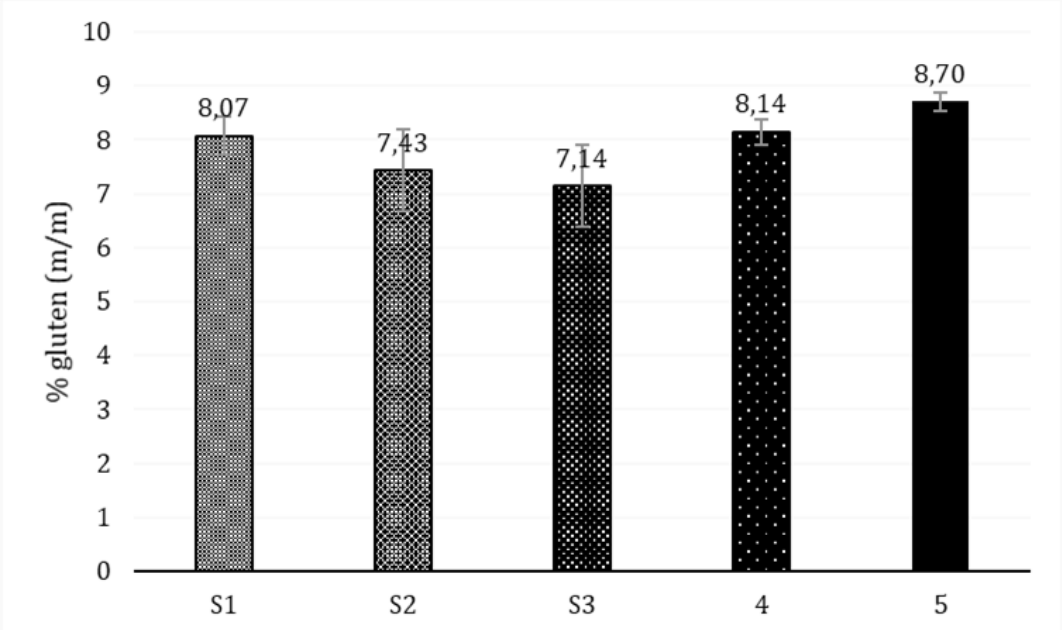

Figure 2. Percentage of dry gluten extracted (m/m) from sourdough doughs and doughs fermented with yeast Saccharomyces cerevisiae.
* Asterisks above the bars indicate significant differences ($p \leq 0.05$) between the samples.
Legend: S1 – Water, wheat flour, and apricot; S2 - Water, wheat flour, and pineapple juice; S3 - Water and wheat flour; 4 - Saccharomyces cerevisiae for 48h; 5 - Saccharomyces cerevisiae for 2h

Identification of LAB with proteolytic potential isolated from sourdough

Nine proteolytic bacterial isolates were obtained from the collected sourdough samples and the results are shown in Table 3.

Table 3
Phenotypic and genotypic identification of LAB isolated from sourdough in medium containing gluten as the sole nitrogen source

SAMPLE	ISOLATES WITH DIFFERENT COLONY MORPHOLOGIES	GRAM	GAS IN GLUCOSE	GROWTH AT 15 ºC	GENOTYPIC
S1	LAB 1	Gram + *bacillus*	negative	positive	*Lactiplantibacillus plantarum*
	LAB 2	Gram + *bacillus*	negative	positive	*Lactiplantibacillus plantarum*
	LAB 8	Gram + *bacillus*	negative	negative	*Lactiplantibacillus plantarum*
S2	LAB 3	Gram + *bacillus*	negative	negative	*Lactiplantibacillus plantarum*
	LAB 4	Gram + *bacillus*	negative	positive	*Companilactobacillus crustorum*
	LAB 5	Gram + *bacillus*	negative	positive	*Lactiplantibacillus plantarum*
	LAB 9	Gram + *bacillus*	negative	positive	*Lactiplantibacillus plantarum*
S3	LAB 6	Gram + *bacillus*	negative	positive	*Companilactobacillus crustorum*
	LAB 7	Gram + *bacillus*	negative	positive	*Companilactobacillus crustorum*

Legend: S1 - Water, wheat flour, and apricot; S2 - Water, wheat flour, and pineapple juice; S3 - Water and wheat flour.

All isolates were phenotypically identified as catalase-negative gram-positive bacilli and showed no gas production during glucose fermentation, which is a characteristic of LAB.

The *16S* rRNA region was used for genotypic identification. This gene is frequently used for LAB identification because of its high level of conservation among these species (Clarridge, 2004). Although Costa et al. (2011) reported the need for an additional

gene to differentiate LAB species, in the present study, it was possible to obtain a distinguishing locus using only this fragment, which not only enabled identification but also allowed differentiation of the isolated genera and species. The isolates were identified as *L. plantarum* and *C. crustorum.*

According to Minervini et al. (2010), the former *Lactobacillus* genus is the most relevant to indigenous sourdough species because of their competitiveness and adaptation to specific environmental conditions. A study conducted by Ravyts and De Vuyst (2011) showed that *L. plantarum* and *C. crustorum* strains produced the highest levels of organic acids compared with that of other LAB, resulting in more pronounced pH reductions in fermented flour/water mixtures and a more sour flavor in the corresponding breads. These characteristics are important because they enhance proteolysis in sourdough and ensure efficient fermentation.

L. plantarum is a versatile species with a heterofermentative metabolism and the ability to adapt to different environmental conditions. It is also one of the three most commonly isolated species of sourdough, particularly those classified as Type I (Di Cagno et al., 2010; Valmorri et al., 2006). This species represents an evolutionary link between homofermentative and heterofermentative LAB. Although *Pediococcus* and *L. plantarum* share key metabolic characteristics with homofermentative bacteria; they are phylogenetically related to the heterofermentative *Lactobacillus* species and *Leuconostocaceae* family (Zheng et al., 2020).

The homofermentative species, *L. crustorum,* now classified as *C. crustorum,* has not been extensively studied and was first recovered from koumiss (fermented mare milk) (Yi et al., 2017; Zheng et al., 2020). Although few studies have reported its isolation from sourdoughs (Comasio et al., 2019; Scheirlinck et al., 2007; Xing et al., 2019; Aktepe et al., 2024), this is believed to have been isolated for the first time in Brazil. Costa et al. (2022) evaluated the microbiota of naturally fermented breads made from organic and conventional wheat flour in southern Brazil. The *Companilactobacillus, Pediococcus, Lactiplantibacillus, Limosilactobacillus, Levilactobacillus, Acetobacter,* and *Gluconobacter* genera were identified. The *Companilactobacillus* sp. was further identified as *Companilactobacillus nuruki.*

The *16S* rRNA gene fragment of the identified *C. crustorum* isolate was deposited in GenBank under accession number OQ629248. The presence of *C. crustorum* as a starter strain in sourdough may lead to increased concentrations of lactic acid, acetoin, and diacetyl, positively affecting the starter and aroma of the fermented products (Comasio et al., 2019). The homofermentative species, *C. crustorum* has also been studied as a probiotic isolated from fermented foods, such as pickles, and is known to produce potent bacteriocins (Sharafi et al., 2015; M. L. Fu & Gu, 2019; Qiao et al., 2021; Aktepe et al., 2024).

Determination of pH and gluten in the fermentation process using isolated LAB

The results regarding the influence of the isolated cultures and yeasts on pH and gluten content in the fermented doughs are

shown in Figure 3; the isolates were named *C. crustorum* (CC), *L. plantarum* (LP), yeast (L1), and yeast after 48 h of fermentation (L2). The bacteria induced a marked decrease in dough pH from the beginning of the experiment, and after 12 h of fermentation, both LAB-treated doughs had already dropped below pH 4.5, with the lowest value observed in the sample inoculated with *C. crustorum.* At the end of the 48-h fermentation, the pH of the dough fermented with *S. cerevisiae* was significantly higher ($p<0.05$) than that of the LAB-inoculated doughs, reaching a minimum value of 5.08, while the samples with *L. plantarum* and *C. crustorum* reached 4.17 and 4.09, respectively.

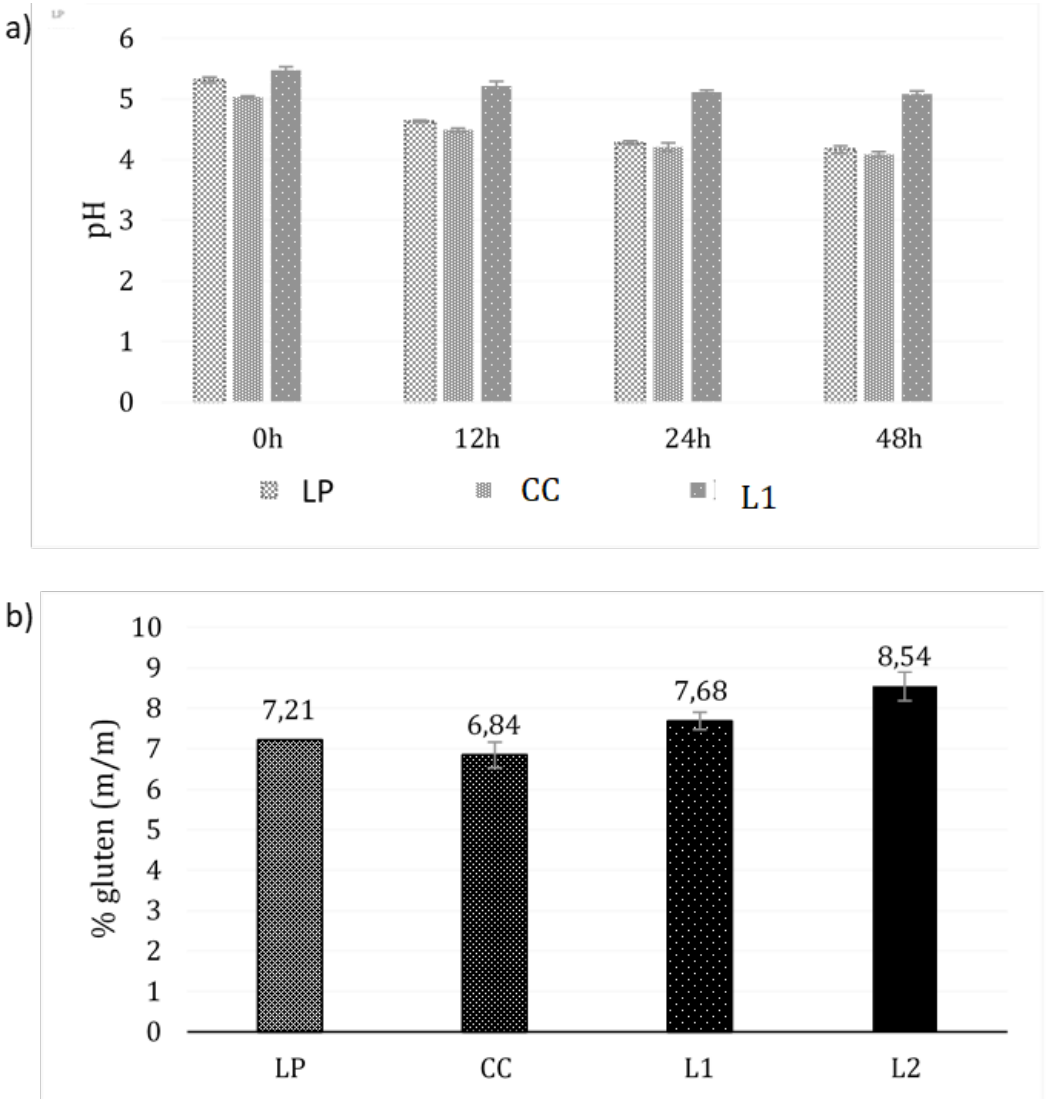

Figure 3. Influence of fermentation time on pH (a) and gluten percentage (b) of doughs fermented with isolated bacteria and those produced with baker's yeast (*S. cerevisiae*).
Legend: LP: *L. plantarum*; CC: *L. crustorum*; L1: *S. cerevisiae* for 48h; L2: *S. cerevisiae* for 2h.

Regarding gluten content, a significant difference ($p<0.05$) was observed when comparing fermentation with *C. crustorum* and yeast, as well as between *L. plantarum, C. crustorum*, and 48-h yeast fermentation, compared to that with the 2-h yeast fermentation. The presence of *C. crustorum* as a starter strain in sourdough, in addition to its ability to increase lactic acid concentrations and positively affect fermentation, as described by Comasio et al. (2019), also appears to enhance wheat protein hydrolysis, resulting in a lower gluten content at the end of fermentation. In a study by Akamine et al. (2023), species such as *L. fermentum, E. faecium, L. plantarum, P. acidilactici, P. pentosaceus,* and *K. unispora* were isolated from sourdoughs and identified as having gluten degradation potential.

Each sourdough starter is unique, with diverse activities, microbial populations, and interactions between yeasts and bactéria resulting from differences in ingredients, environment, and fermentation time (Lau et al., 2021) therefore, studies, such as the present one are important for the isolation of new species with proteolytic potential that can serve as valuable starter cultures for producing breads with lower levels of allergenic proteins. However, further studies, such as immunological assays, proteomic analyses, and clinical trials, are required to evaluate this reduction in allergenicity.

Conclusion

The impact of natural sourdough fermentation was compared with conventional fermentation, revealing greater acidification and gluten reduction in sourdough samples. The *L. plantarum* and *C. crustorum* species were isolated and identified as potential starter cultures. Although sourdough does not completely eliminate gluten, it can reduce gluten content and improve the digestibility of wheat-based products.

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