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RESEARCH ARTICLE



Reducing FODMAPs and improving bread quality using type II sourdough with selected starter cultures

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ABSTRACT

This study focussed on lactic acid bacteria (LAB) screening for sourdough type II elaboration and evaluating the effects of sourdough fermentation in bread making, focussing mainly on reducing FODMAPs. After a technological performance screening, six strains (*Levilactobacillus brevis*, *Weissella minor*, *Lactiplantibacillus plantarum*, *Leuconostoc citreum*, *Limosilactobacillus fermentum*, and *Companilactobacillus farciminis*) were selected for sourdough preparation. Total titratable acidity, pH, specific volume, and enumeration of microorganisms were carried out on sourdoughs, doughs, and breads. Breads were subjected to texture profile and colour analysis, moulds and yeast enumeration, and total fructans (main group of FODMAPs) quantification. Breads produced with sourdough showed a significant reduction of fructans, greater acidity, volume, and better performance during storage when compared to fermentation using only baker's yeast. Including specific cultures as starters in sourdough reduced fructans content by >92%, thereby producing a low FODMAP bread suitable for Irritable Bowel Syndrome patients with improved nutritional and technological properties.

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syndrome; texture
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Introduction

Over millennia, the production of bread remained essentially dependent on the artisanal sourdough, a dough of wheat flour and water, spontaneously fermented by lactic acid bacteria (LAB) and yeasts. This long, laborious, and uncontrolled process from a microbiological point of view, characterises traditional or type I sourdough (Chavan and Chavan 2011; Gobetti et al. 2014; Siepmann et al. 2018). The need to accelerate the fermentation process from the 20th century onwards led the bakery industry to replace traditional sourdough almost entirely with baker's yeast. However, nowadays, the growing consumer interest in natural fermentation has directed industrial production towards the use of type II sourdough, in which selected LAB are intentionally added to the dough as a starter. This makes it possible to reduce sourdough preparation time, increase microbiological

safety, and standardise bread quality (Siepmann et al. 2018; Brandt 2019).

Sourdough can provide several benefits to the nutritional, sensory, and technological quality of the products in which it is applied. Its importance is mainly associated with the ability of LAB to originate organic acids, exopolysaccharides, aromatic, and anti-fungal/antibacterial. Consequently, the production of these compounds during fermentation can increase the shelf life of bread, improve the texture and flavour properties, increase the amounts of bioactive compounds (Arendt et al. 2007; Flander et al. 2011; Nionelli and Rizzello 2016; Demirbaş et al. 2017; Gobetti et al. 2019). LAB capable of degrading the complex network formed by wheat proteins are also interesting by making it possible to obtain bread with reduced gluten content, reduced in allergenic fractions of wheat proteins or immunogenic epitopes (De Angelis et al. 2010; Francavilla et al. 2017), increased

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in free peptides and amino acids, aromatic compounds, and bioactive peptides (Poutanen et al. 2009; Taylan et al. 2019).

Pioneering studies regarding the ability of type I sourdough to partially reduce the concentration of all carbohydrates classified as FODMAPs (Fermentable Oligo-, Di-, Mono-Saccharides And Polyols) in bread, have recently been published (Menezes et al. 2018, 2019). FODMAPs are a class of carbohydrates, most of them short-chain compounds that in some individuals can trigger the symptoms of Irritable Bowel Syndrome (IBS) and Non-Celiac Gluten Sensitivity (NCGS) (Böhn et al. 2015). Several studies have shown that a diet reduced in FODMAPs can relieve the symptoms of IBS and NCGS (Ziegler et al. 2016). Bakery products are the biggest sources of FODMAPs in the Western diet (Verspreet et al. 2015) therefore, breads with reduced concentrations of FODMAPs are great alternatives for consumers with these specific nutritional needs.

The use of a type II sourdough, composed of selected and well-characterized LAB, can contribute to overcoming natural fermentation challenges. Furthermore, the selection of LAB strains is interesting for the development of sourdough to obtain wheat bread with improved technological and nutritional properties. Therefore, this study explored the technological aptitude of LAB strains in order to select those with desired performance and combine them in co-culture to produce different type II sourdoughs. These were used in bread making and compared to conventional fermentation by baker's yeast with the main objective of obtaining low-FODMAPs bread.

Materials and methods

Flow-chart of the work with the LAB screening, type II sourdough production, and bread making can be visualised in Figure 1.

Microorganisms

Fourteen strains of LAB (Table 1) belonging to the Food and Bioprocess Technology Laboratory of the Federal University of Santa Catarina (UFSC, Brazil), kept under freezing (-20°C) in microtubes containing Man-Rogosa-Sharpe broth (MRS, Sigma-Aldrich, St. Louis, Missouri, USA) and glycerol (Quimibrás S.A, Rio de Janeiro, Rio de Janeiro, Brazil) (20% v/v), were recovered in MRS broth (Sigma-Aldrich) at 37°C for 24 h in an anaerobic jar with Anaerocult (Merck, Darmstadt, Hesse, Germany). The recovery

process was repeated two more consecutive times. Subsequently, the strains were centrifuged at 4000-g for 10 min at 4°C and the precipitate from each culture was resuspended in buffered peptone water (BPW) (Oxoid, Basingstoke, Hampshire, United Kingdom) (0.01% w/v) and applied in technological aptitude screening.

Technological aptitude screening

Potential of acidification

The ability of LAB to acidify different media was evaluated according to the adapted methodology of Manini et al. (2016). Reconstituted skimmed milk (RSM) (Molico, Nestlé, São Paulo, São Paulo, Brazil) (10 g of RSM/100 ml of water) and wheat flour (WF) (Ecobio, Coronel Bicaco, Rio Grande do Sul, Brazil) broth (3 g of wheat flour/100 mL of water) were autoclaved at 121°C for 15 min. LAB recovered as described previously, resuspended in BPW were inoculated (1% v/v) in RSM or WF media and incubated at 37°C . The pH was determined at 2 h intervals until completing 12 h of fermentation, and thereafter every 6 h until completing 24 h. All samples were analysed in a technical triplicate.

Carbohydrate metabolic preference and carbon dioxide (CO_2) production

The carbohydrate metabolic preference was determined using a modified MRS medium (Sigma-Aldrich) supplemented with different carbohydrates, according to the methodology adapted from Manini et al. (2016). The strains were inoculated at a concentration of 5 log CFU/mL and incubated at 37°C for 24 h in aerobiosis. For the evaluation of CO_2 production, the strains were inoculated in 9 mL of MRS broth (Sigma-Aldrich) containing Durham tube and incubated in anaerobiosis (Anaerocult) at 37°C for 48 h.

Exopolysaccharide production

LAB strains were inoculated (5 log CFU/mL) on MRS medium plates supplemented with a single carbon source (sucrose or maltose in a concentration of 40 g/L). The plates were incubated in anaerobiosis (Anaerocult) for 48 h at 25, 30, or 37°C . The activity of the strains was classified as the production of EPS, intense production, or absence of production according to a method adapted from Bancalari et al. (2019).

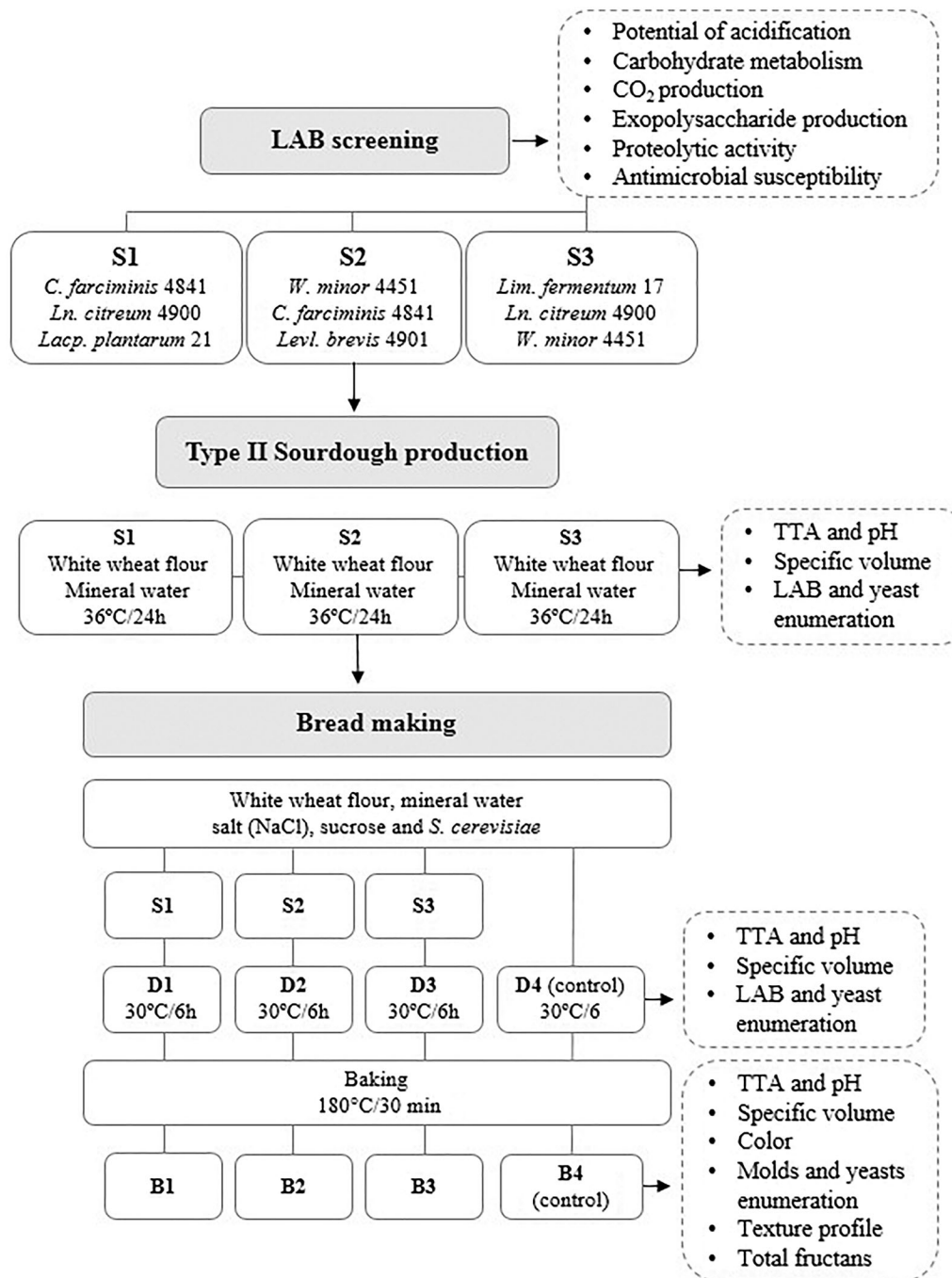


Figure 1. Flow-chart of the work with the lactic acid bacteria (LAB) screening, type II sourdough production, and bread making. S1, S2, and S3: sourdoughs; TTA: total titratable acidity; D1, D2, D3, and D4: doughs; B1, B2, B3, and B4: breads.

Proteolytic activity

The presumable proteolytic capacity was evaluated according to the methodology proposed by Helmerhorst and Wei (2014), with adaptations. The strains were streaked (5 log CFU/mL) on agar plates containing gluten (GA) (Selettogrão, São Paulo, São Paulo, Brazil) or calcium caseinate (CCA) (Merck, Darmstadt, Hesse, Germany), in a concentration of 23 g/L of each one. The plates were incubated in anaerobiosis (Anaerocult) at 37 °C for 24 h. As a result, the

formation of bacterial colonies was observed. The experiment was carried out in triplicate.

Antimicrobial susceptibility

The susceptibility to clinical antimicrobials was evaluated by the disc diffusion method in MRS medium (Sigma-Aldrich), with 6 mm diameter discs, containing antibiotics: erythromycin (15 µg), ampicillin (10 µg), vancomycin (30 µg), streptomycin (10 µg),

Table 1. Lactic acid bacteria strains and fermentative classification.

Microorganism	Strain	Source	Classification*
<i>Companilactobacillus farciminis</i>	LBP UFSC 4841	Sourdough	Homo
<i>Lactacaseibacillus casei</i>	LBP UFSC 19	Unknown	FHete
<i>Lactacaseibacillus paracasei</i>	LBP UFSC 13	Unknown	FHete
<i>Lactacaseibacillus rhamnosus</i>	ATCC 7469	Unknown	FHete
<i>Lactiplantibacillus plantarum</i>	LBP UFSC 21	Unknown	FHete
<i>Lactobacillus acidophilus</i>	LBP UFSC 18	Sourdough	Homo
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	LBP UFSC 1	Cheese	Homo
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	LBP UFSC 15	Cheese	Homo
<i>Lactobacillus helveticus</i>	LBP UFSC 16	Unknown	Homo
<i>Leuconostoc citreum</i>	LBP UFSC 4900	Sourdough	OHete
<i>Levilactobacillus brevis</i>	LBP UFSC 4901	Sourdough	OHete
<i>Limosilactobacillus fermentum</i>	LBP UFSC 17	Unknown	OHete
<i>Pediococcus pentosaceus</i>	ATCC 33316	Beer	Homo
<i>Weissella minor</i>	LBP UFSC 4451	Sourdough	OHete

*Homo: homofermentative; FHete: facultative heterofermentative; OHete: obligate heterofermentative.

chloramphenicol (30 µg), clindamycin (2 µg), tetracycline (30 µg), or gentamicin (10 µg) (Laborclin, Pinhais, Paraná, Brazil). Blank discs were used as a negative control. The zones of inhibition around the discs were measured after incubation in anaerobiosis (Anaerocult) at 37 °C for 24 h and the strains were classified as resistant (R), moderately susceptible (MS), or susceptible (S), based on the reference values indicated by Charteris et al. (1998).

Sourdough production and bread making

After the technological aptitude screening, six strains were selected, namely: *Companilactobacillus farciminis* 4841 (formerly *Lactobacillus farciminis*), *Leuconostoc citreum* 4900, *Lactiplantibacillus plantarum* 21 (formerly *Lactobacillus plantarum*), *Weissella minor* 4451, *Levilactobacillus brevis* 4901 (formerly *Lactobacillus brevis*), *Limosilactobacillus fermentum* 17 (formerly *Lactobacillus fermentum*) for the preparation of three type II sourdoughs (S1, S2, and S3). The cultures were centrifuged at 4000-g for 5 min, washed twice in BPW (0.01% w/v), and resuspended in sterile water. Three LAB were combined to form the starter pool – S1: *C. farciminis* 4841, *Ln. citreum* 4900, and *Lacp. plantarum* 21; S2: *W. minor* 4451, *C. farciminis* 4841, and *Levl. brevis* 4901; S3: *Lim. fermentum* 17, *Ln. citreum* 4900, and *W. minor* 4451. The sourdoughs were prepared from a mixture of white organic wheat flour (Ecobio) and mineral water 1:1 (w/v) in triplicate, with the respective starter in a concentration of 8–9 log CFU/g in the fermented doughs, according to the optical density measurement. The doughs were incubated at 36 °C for 24 h.

The pH value of doughs was determined by a pH metre (Del Lab, Araraquara, São Paulo, Brazil). Total titratable acidity (TTA) was determined according to

AACC (2010). Measurements were taken at 0 h and after fermentation (24 h). The specific volume was determined by the seed displacement method, as described by Hallén et al. (2004) with adaptations. A dough fraction (10 g) was inserted into a graduated cylinder. The graduated cylinder was topped up with rapeseed. The volume of the rapeseed was noted. Another 10 g aliquot was inserted in another graduated cylinder and left to ferment. After 24 h, the empty space of the graduated cylinder was filled with rapeseed and the volume was noted. The calculation was performed as described in the reference. LAB and yeasts were enumerated on MRS medium (Sigma-Aldrich) supplemented with ciclopirox olamine (10 mg/mL) and Yeast Extract, Peptone, and Glucose medium (YEPG, Himedia, Mumbai, India) supplemented with chloramphenicol (Sigma-Aldrich, St. Louis, Missouri, USA) (10 mg/mL), respectively, at 0 h and after fermentation (24 h).

Four doughs (D1, D2, D3, and D4) were formulated with 600 g of white organic flour (Ecobio), 300 g of mineral water, 13.8 g of salt (NaCl), 15 g of sucrose, and 6 g of *Saccharomyces cerevisiae* baker's yeast (Fleischmann, São Paulo, São Paulo, Brazil). Formulations D1, D2, and D3 were supplemented with 15% (w/w; on the weight of the flour) of sourdough S1, S2, and S3, respectively. One control treatment (D4) was formulated only with baker's yeast. The doughs were fermented for 6 h in an incubator at 30 °C and baked at 180 °C for 30 min in an oven with forced air circulation and steam injection (Venâncio, Venâncio Aires, Rio Grande do Sul, Brazil). The doughs were evaluated for pH and TTA, LAB and yeast count, and specific volume as previously described, in technical triplicate, at 0 and 6 h of fermentation. After baking, the breads (B1, B2, B3, and B4) were stored in polyethylene plastic bags, at room temperature, for 5 days while the analyzes were performed.

Bread analysis

The breads were evaluated for pH and TTA as described in the section above. Moulds and yeasts were enumerated by plating and incubation at 25 °C for 5 days on Potato Dextrose Agar (PDA; Merck, Darmstadt, Hesse, Germany) acidified with a sterile tartaric acid (Hedy Química, São Paulo, São Paulo, Brazil) solution (10% v/v) until the pH 3.5 was reached. The texture profile was obtained using a texture analyser (model TA.HD plus, Stable Micro Systems, Godalming, Surrey, United Kingdom) according to Aplevicz et al. (2014). These analyses were performed on the first and fifth day of storage. The specific volume of the breads was determined as described in the section above on the first day after baking.

Total fructans were quantified using the enzymatic test kit Megazyme Fructan HK (Megazyme, Bray, Leinster, Ireland) according to the manufacturer's instructions. Sorbitol and mannitol were quantified in a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (Sciex, Framingham, Massachusetts, USA) equipped with an electrospray ionisation (ESI) source according to Menezes et al. (2019). The concentration was determined as the sum of the isomers sorbitol and mannitol (SOR/MAN) since it was not possible to separate them under the chromatographic tested conditions. All determinations were carried out in triplicate.

Colour determination of the breadcrumb was evaluated using the colorimeter Chroma Metre (CR-400, Konica Minolta Sensing Inc., Tokyo, Japan). To calculate the colour parameters L*, a*, b*, c*, and H, the CIELab colour scale was used (Aplevicz et al. 2014).

Statistical analysis

The data were statistically analysed employing the software Statistica® version 13.3 and RStudio version 3.6.0 "Planting of a Tree", with 95% confidence interval. For the texture profile the non-parametric statistical test Kruskal–Wallis was used. The Conover-Iman multiple comparisons test was used to assess statistical differences between samples.

Results and discussion

Technological aptitude screening and susceptibility

The LAB strains that demonstrated the best acidifying capacity in RSM were *Lactobacillus bulgaricus* 1, *L. bulgaricus* 15, *W. minor* 4451, and *Lactobacillus helveticus* 16, which reduced the pH below 4.3 in 24 h. In the WF medium, the LAB that demonstrated the best acidifying capacity were *Lactobacillus acidophilus* 18, *Levl. brevis* 4901, *W. minor* 4451 (pH below 4.5 in 24 h), and *Lacp. plantarum* 21 (pH 4.15 in 24 h). In a similar study, Manini et al. (2016) observed that *Lacp. plantarum* showed the highest acidification rate in wheat bran dough. *W. minor* 4451, *Lacticaseibacillus casei* 19 (formerly *Lactobacillus casei*), and *L. bulgaricus* showed the ability to metabolise a greater diversity of sugars (Table 2). Only *Lacticaseibacillus paracasei* 13 (formerly *Lactobacillus paracasei*), and *Levl. brevis* 4901 were able to produce CO₂ in 48 h.

Five strains demonstrated the ability to produce EPS. The largest producers were *Ln. citreum* 4900, *P. pentosaceus* ATCC 33316, and *Lacticaseibacillus rhamnosus* ATCC 7469 (formerly *Lactobacillus rhamnosus*).

Table 2. Carbohydrate metabolic preference and exopolysaccharides (EPS) production by lactic acid bacteria strains in MRS medium containing sucrose (Suc), trehalose (Tre), fructose (Fru), lactose (Lac), maltose (Mal), mannose (Man), starch (Sta), raffinose (Raf), rhamnose (Ram), or sorbitol (Sor).

Microorganism	Carbohydrate										EPS					
	Suc	Tre	Fru	Lac	Mal	Man	Sta	Raf	Rha	Sor	Suc 25 °C	Suc 30 °C	Suc 37 °C	Mal 25 °C	Mal 30 °C	Mal 37 °C
<i>C. farciminis</i> LBP UFSC 4841	+	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+
<i>Lact. casei</i> LBP UFSC 19	+	++	+	++	++	+	++	+	+	+	–	+	+	–	+	+
<i>Lact. paracasei</i> LBP UFSC 13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Lcb. rhamnosus</i> ATCC 7469	++	++	++	++	–	++	+	+	+	+	++*	++	+	++	++	++*
<i>Lacp. plantarum</i> LBP UFSC 21	+	+	+	+	+	++	++	+	+	+	+	++	+	+	++	+
<i>L. acidophilus</i> LBP UFSC 18	–	–	+	+	+	–	++	–	–	–	+	+	+	=	+	+
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> LBP UFSC 1	+	++	+	+	+	+	++	++	+	+	–	+	+	–	+	+
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> LBP UFSC 15	+	++	++	++	+	++	++	+	+	++	+	+	–	+	+	–
<i>L. helveticus</i> LBP UFSC 16	+	+	+	+	+	+	+	–	–	+	–	+	+	–	+	+
<i>Ln. citreum</i> LBP UFSC 4900	+	+	+	+	+	+	+	+	+	+	++	++	+	+	+	+
<i>Levl. brevis</i> LBP UFSC 4901	+	+	+	+	+	+	++	+	+	+	+	++	+	+	+	+
<i>Lim. fermentum</i> LBP UFSC 17	+	–	+	+	+	–	+	+	–	–	+	+	+	+	+	+
<i>P. pentosaceus</i> ATCC 33316	+	+	+	+	+	+	+	+	+	+	++	++	+	++	++*	+
<i>W. minor</i> LBP UFSC 4451	++	++	++	++	++	+	++	++	+	+	–	+	+	–	+	+

Results: + growth/production; ++ intense growth/production; – absence of growth/absence of production; *expressive production.

Table 3. Antimicrobial susceptibility profile of lactic acid bacteria strains obtained by disc diffusion method.

Susceptibility level (halo diameter in mm)		L. delbrueckii																W. minor								
Concentration (µg)	Moderately Resistant	Susceptible	C. farcinimis		Lact. casei		Lact. paracasei		Lcb.		Lacp.		L. acidophilus		subsp. bulgaricus		L. helveticus		Ln.		Levl.		Lim.		P. pentosaceus	LBP UFSC 4451
			LBP UFSC 4841	UFSC 19	LBP UFSC 19	UFSC 13	ATCC 7469	LBP UFSC 21	LBP UFSC 18	LBP UFSC 15	LBP UFSC 16	LBP UFSC 17	LBP UFSC 16	LBP UFSC 15	LBP UFSC 16	LBP UFSC 17	LBP UFSC 16	LBP UFSC 15	LBP UFSC 16	LBP UFSC 17	LBP UFSC 16	LBP UFSC 15	LBP UFSC 16	LBP UFSC 17		
Antimicrobial																										
Ampicillin	10	≤12	13–15	≥16	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	MS
Erythromycin	15	≤13	14–17	≥18	MS	R	S	S	S	S	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S	R
Tetracycline	30	≤14	15–18	≥19	R	R	MS	S	S	S	S	S	R	S	S	S	S	S	S	R	S	R	S	S	S	R
Streptomycin	10	≤11	12–14	≥15	R	R	R	S	S	S	R	R	R	S	R	R	S	S	S	S	R	R	MS	R	R	R
Gentamicin	10	≤12	–	≥13	S	R	R	S	S	S	S	S	R	S	R	R	S	R	S	S	S	S	S	S	S	R
Clindamycin	2	≤8	9–11	≥12	S	R	R	S	S	S	S	S	R	S	R	R	S	S	S	S	S	S	S	S	S	S
Vancomycin	30	≤14	15–16	≥17	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	S
Chloramphenicol	30	≤13	14–17	≥18	S	R	S	S	S	S	S	S	R	S	R	R	S	S	S	S	S	S	S	S	S	R

Changes of the zone of inhibition diameters exhibited by bacteria considered susceptible (S), moderately susceptible (MS), or resistant (R) to each antibiotic.

Ranges of the zone of inhibition diameters exhibited by bacteria considered susceptible (S), moderately susceptible (MS), or resistant (R) to each antibiotic.

Among them, *Ln. citreum* 4900 had the most expressive EPS production in sucrose at 25 and 30 °C (Table 2). The production of EPS by LAB can improve the rheological characteristics of the sourdough, final texture, and extend the shelf life of the bread, in addition to being used to replace or reduce the use of more expensive hydrocolloids, a potential to be exploited by industry (Galle and Arendt 2014).

All strains surveyed were capable to grow in CCA, an indication that are able to consume calcium caseinate as a source of nitrogen. However, only *L. bulgaricus* 1, *L. bulgaricus* 15, *L. helveticus* 16, *W. minor* 4451, and *Lcb. rhamnosus* ATCC 7469 were able to grow in GA, therefore, they are presumably able to degrade gluten. Growing on GA agar is a first step in selecting strains capable of utilising gluten. Notwithstanding, strains that grow well on GA agar need further evaluation to be considered effective in reducing gluten content or specific gluten fractions in products.

For the antimicrobial susceptibility test (Table 3), all strains showed resistance to at least one antibiotic. Although not a direct effect on safety, some microorganisms, including species of the Lactobacillaceae associated with fermented foods, can carry transmissible antibiotic-resistance genes (Campedelli et al. 2018). This is in line with other reports, indicating that some resistances appear to be intrinsic for lactobacilli. In the work of Hummel et al. (2007), more than 70% of the starter and probiotic strains of LAB were resistant to gentamicin, streptomycin, and ciprofloxacin. The transfer of resistance genes is common among these microorganisms, a skill used for survival and environmental adaptation (Herrerros et al. 2005). The tests carried out in this study are only indicative and would need to be complemented at the genotype level, to find out if resistance genes are horizontally transmissible or if they are intrinsic genes to the species. The two strains of *L. bulgaricus* (1 and 15) were resistant to all antibiotics tested. For this reason, although presented good results for technological aptitude tests, they were not selected for bread making.

According to the responses obtained regarding the technological aptitude profile, *Levl. brevis* 4901, *W. minor* 4451, *Lacp. plantarum* 21, *Ln. citreum* 4900, *Lim. fermentum* 17, and *C. farciminis* 4841 were selected as starters for the sourdoughs.

Sourdough fermentation and bread making

For all sourdoughs samples, there was a significant reduction in pH and TTA ($p < 0.05$) after 24 h of

Table 4. Total titratable acidity (TTA), pH, microbial counts for lactic acid bacteria (LAB) and yeasts (log CFU/g), total fructans (g/100 g), sorbitol and mannitol (SOR/MAN; mg/100 g) for sourdough at time 0 and 24 h, dough at times 0 and 6 h, and bread at 1 and 5 days.

Sourdough (S)				
	S1	S2	S3	
pH _{0h}	6.34 ± 0.04 ^{aA}	6.31 ± 0.02 ^{aA}	6.22 ± 0.01 ^{aA}	
pH _{24h}	3.74 ± 0.01 ^{cB}	3.92 ± 0.01 ^{aB}	3.77 ± 0.02 ^{bB}	
TTA _{0h}	2.13 ± 0.47 ^{aB}	1.01 ± 0.10 ^{bB}	2.02 ± 0.10 ^{aB}	
TTA _{24h}	14.77 ± 2.57 ^{aA}	11.33 ± 0.82 ^{aA}	12.87 ± 0.41 ^{aA}	
LAB _{0h}	8.97 ± 0.96 ^{aA}	10.34 ± 0.55 ^{aA}	9.45 ± 0.52 ^{aB}	
LAB _{24h}	11.56 ± 1.15 ^{aA}	10.99 ± 0.37 ^{aA}	12.57 ± 0.62 ^{aA}	
Yeast _{0h}	<4.00 ± 0.01 ^{bA}	4.33 ± 0.53 ^{aA}	3.62 ± 2.30 ^{aA}	
Yeast _{24h}	<4.00 ± 0.01 ^{bA}	5.88 ± 0.83 ^{aA}	6.71 ± 0.12 ^{aA}	
Dough (D)				
	D1	D2	D3	D4
pH _{0h}	5.15 ± 0.01 ^{cA}	5.20 ± 0.01 ^{bA}	5.19 ± 0.02 ^{bA}	5.59 ± 0.01 ^{aA}
pH _{6h}	4.33 ± 0.02 ^{cB}	4.73 ± 0.04 ^{bB}	4.44 ± 0.02 ^{cB}	5.37 ± 0.03 ^{aB}
TTA _{0h}	2.85 ± 0.18 ^{aB}	2.49 ± 0.31 ^{aA}	2.73 ± 0.37 ^{aB}	1.36 ± 0.10 ^{bB}
TTA _{6h}	3.86 ± 0.10 ^{aA}	3.56 ± 0.71 ^{aA}	3.74 ± 0.01 ^{aA}	2.43 ± 0.21 ^{bA}
LAB _{0h}	10.08 ± 0.22 ^{aA}	10.47 ± 0.52 ^{aA}	11.24 ± 0.48 ^{aA}	2.00 ± 0.01 ^{bB}
LAB _{6h}	10.05 ± 0.08 ^{aA}	10.83 ± 0.30 ^{aA}	10.44 ± 0.94 ^{aA}	5.60 ± 0.05 ^{bA}
Bread (B)				
	B1	B2	B3	B4
pH _{first day}	4.51 ± 0.01 ^{cA}	4.66 ± 0.02 ^{bA}	4.46 ± 0.04 ^{cA}	5.59 ± 0.01 ^{aA}
pH _{fifth days}	4.42 ± 0.03 ^{cB}	4.66 ± 0.05 ^{bA}	4.44 ± 0.05 ^{cA}	5.54 ± 0.03 ^{aB}
TTA _{first day}	4.92 ± 0.21 ^{aA}	4.33 ± 0.10 ^{bA}	5.22 ± 0.11 ^{aA}	2.81 ± 0.28 ^{cA}
TTA _{fifth days}	5.45 ± 0.45 ^{aA}	4.39 ± 0.27 ^{bA}	5.39 ± 0.11 ^{aA}	3.16 ± 0.13 ^{cA}
Yeast/mold _{first day}	2.74 ± 1.04 ^{cA}	<2.00 ± 0.01 ^{bB}	3.45 ± 0.21 ^{aB}	3.93 ± 0.03 ^{aB}
Yeast/mold _{fifth days}	3.50 ± 0.28 ^{cA}	4.72 ± 0.13 ^{bA}	5.39 ± 0.15 ^{aA}	5.74 ± 0.12 ^{aA}
Fructans _{first day}	0.17 ± 0.14 ^b	0.06 ± 0.04 ^b	0.24 ± 0.04 ^b	0.81 ± 0.15 ^d
SOR/MAN _{first day}	31.55 ± 0.17 ^a	15.94 ± 0.30 ^c	20.81 ± 0.00 ^b	13.53 ± 0.07 ^d

S1: *Companilactobacillus farcinis* 4841, *Leuconostoc citreum* 4900, and *Lactiplantibacillus plantarum* 21; S2: *Weissella minor* 4451, *C. farcinis* 4841, and *Levilactobacillus brevis* 4901; S3: *Limosilactobacillus fermentum* 17, *Ln. citreum* 4900, and *W. minor* 4451.

D1: formulated with S1; D2: formulated with S2; D3: formulated with S3; D4: control formulated only with baker's yeast.

B1: D1 baked; B2: D2 baked; B3: D3 baked; B4: D4 baked.

Values followed by the same letter, uppercase in the column (^{A,B}) and lowercase in the row (^{a,b,c}), do not differ significantly by the Tukey test ($p > 0.05$).

TTA: ml of 0.1 M NaOH/10 g of dough.

fermentation. S1 showed greater acidification capacity, expressed by the pH and TTA values, followed by S3. After 24 h, the LAB starters of S1, S2, and S3 were able to significantly reduce pH and increase TTA to values similar to those reported for mature sourdoughs – pH range between 3.4 and 4.9 and TTA with a median value of 11.0 ml of 0.1 M NaOH/10 g of dough (Arora et al. 2021) (Table 4).

The greater acidification capacity of sourdough S1 and S3 became more evident, since the pH values were significantly lower ($p < 0.05$) for D1 and D3. All sourdoughs showed greater acidification capacity compared to the control treatment (D4), since the last one showed statistically lower TTA and higher pH ($p < 0.05$). The same behaviour was observed for breads after baking (B1 and B3), evidencing the

greater acidifying capacity of S1 and S3 and the low capacity for converting carbohydrates into organic acids presented by baker's yeast, as expected, considering that expressive pH reduction is not a characteristic of yeasts (De Vuyst et al. 2016). The pH values found for sourdough were similar to other studies already reported (Nionelli et al. 2014; Yu et al. 2018), showing the good acidifying capacity of the selected cultures.

Only for S3, there was a significant increase ($p > 0.05$) in the number of viable and cultivable LAB cells during the 24 h of fermentation. There was no statistically significant difference ($p > 0.05$) between doughs D1, D2, and D3 regarding the LAB counts. However, D4 differed statistically from the other treatments ($p < 0.05$), presenting the lowest counts (Table 4). Values between 8 and 9 log CFU/g for LAB, 6 and 7 log CFU/g for yeasts are expected for mature sourdoughs (Nionelli et al. 2014; Palla et al. 2017). Therefore, S1, S2, and S3 were in an appropriate state of development. Thus, it is evident that the careful selection of LAB starters can enhance the production of sourdough in a short time.

B4 showed a statistically higher concentration of fructans ($p < 0.05$) than samples produced with sourdough, which in turn did not differ from each other (Table 4). The reduction was 92.6, 79.0, and 70.4%, for B2, B1, and B3, respectively, concerning the total fructans content for B4. Fructans are considered to be the largest component of the FODMAPs group (Menezes et al. 2018). Menezes et al. (2019) found an average reduction of 72% compared to bread made with baker's yeast. This reduction is attributed to the ability of some LAB to express specific enzymes for degradation of fructans. In doughs fermented by bacteria and yeasts, LAB create acidic conditions that improve yeast invertase activity, which may further increase the degradation (Nilsson et al. 1987). As LAB can synthesise mannitol from fructans, mannitol levels should be determined in sourdough bread intended for IBS-sufferers (Vrancken et al. 2011). Although there was an increase in the concentration of SOR/MAN for the sourdough bread samples, these compounds were produced in lower amounts (0.01–0.03 g/100 g) in relation to fructans, so does not contribute significantly to increasing the total concentration of FODMAPs. Fructans levels for the sourdough bread samples are below the cut-off value (<0.3 g oligosaccharides/serving for grains or cereals) for low FODMAP diet that was defined by Varney et al. (2017) for IBS patients. Thus, the sourdough breads obtained are more suitable for IBS patients and better tolerated than baker's yeast bread.

Table 5. Colour parameters (L^* , a^* , b^* , c^* , and H) for the breadcrumb on the first day of storage.

Bread	L^*	a^*	b^*	c^*	H
B1	66.63 ± 1.16 ^A	1.49 ± 0.26 ^A	1.54 ± 0.82 ^A	17.61 ± 0.83 ^A	85.40 ± 0.41 ^A
B2	65.93 ± 1.34 ^A	1.29 ± 0.25 ^A	17.01 ± 0.90 ^A	17.07 ± 0.91 ^A	85.67 ± 0.68 ^A
B3	65.62 ± 2.94 ^A	1.25 ± 0.07 ^A	16.83 ± 0.53 ^A	16.87 ± 0.54 ^A	85.75 ± 0.29 ^A
B4	65.78 ± 1.86 ^A	1.29 ± 0.08 ^A	17.37 ± 0.50 ^A	17.24 ± 0.48 ^A	85.04 ± 0.57 ^A

Values in the same column, followed by the same uppercase letter (^A), do not differ significantly by Tukey test ($p > 0.05$).

There was no minimum statistical difference ($p > 0.05$) between samples for analysed colour parameters (Table 5).

The specific volume for all doughs in 0 h was 1.23 g/mL. After 6 h of fermentation, D4 apparently exhibited a smallest increase in specific volume. The values were 3.33, 3.00, 2.80, and 2.66 g/mL, for D1, D2, D3, and D4, respectively. The specific volume obtained for type II sourdough is one of the main parameters used to evaluate the production and retention of CO₂ synthesised by LAB and yeasts present (Arendt et al. 2007). The fermentation is an important step to obtain bread with the desired textural properties. In that regard, the change in the dough volume is an indicator for microbial activity and bread quality since the higher the specific volume, the more airy and soft it becomes (Vernon-Carter et al. 2017). Although CO₂ is a metabolite synthesised by yeasts, the increase in the specific volume in sourdough can be attributed to the ability of LAB to enhance the formation of this gas. Yu et al. (2018) reported that sourdough improves the expansion capacity of the dough, not only because of the higher CO₂ production, but also because the acidic environment contributes to the retention of gases by the gluten network.

Bread analysis

For pH values, a significant decrease was observed only for B1 and B4 breads during 5 days of storage. On the fifth day of storage, B1 and B3 had the lowest pH values, while B4 had the highest value, differing significantly from the others ($p < 0.05$). For TTA, there was no significant variation during the evaluated period, B4 remaining with the lowest, and B1 and B3 with the highest value (Table 4).

According to Arora et al. (2021), staling and fungal contamination are the main causes of loss of bread quality during shelf life of baked goods. Only for B1, there was no significant increase ($p > 0.05$) in the count of moulds and yeasts during the storage period. For B2, although a significant increase in the count was observed, the development of these

microorganisms was lower than that of B3 and B4. On the fifth day of storage, B4 showed the highest moulds and yeasts count and, although no statistical difference from the B3 count was detected, only in the B4 was the development of fungi visually observed (data not shown).

Numerous studies have reported the ability of sourdough to delay the growth of moulds, therefore, the addition of sourdough may be an alternative for the food industry to extend the shelf life of bread, without the addition of preservatives (Ryan et al. 2008; Garofalo et al. 2012). The antifungal activity of sourdough is mainly correlated with lactic and acetic acids produced by LAB (Debonne et al. 2020).

The texture profile of the breads during the storage period is shown in Table 6. For the springiness parameter, no bread samples differed significantly ($p > 0.05$). In contrast, the results for resilience and cohesiveness variables showed significant differences ranging from 19.80% (B3, fifth day) to 35.18% (B4, first day), and 0.48 (B3, fifth day) up to 0.72 (B4, first day), respectively. When comparing the values of a single treatment between the first and the fifth day all results showed statistical difference ($p > 0.05$), which demonstrates that after five days the breads showed less resilience, cohesiveness, and springiness regardless of the baker's yeast used.

For the parameters gummy, chewiness, and hardness, a non-parametric statistical test was used (Table 7). The analysis of variance by the Kruskal-Wallis test for gummy, chewiness, and hardness variables indicated significant differences among samples ($p < 0.05$). The Conover-Iman multiple comparisons test was used to assess statistical differences between samples. On the first day, breads did not differ significantly in terms of gummy. However, samples B2, B3, and B4 of the first day showed significant differences to B4 and B5 of the fifth day, as well as B2 and B4 of the fifth day, as B4 showing the highest median. Besides, there was also a significant increase in the gummy results for the B4 bread during storage.

For the chewiness parameter, results for B4 of the fifth day showed the highest value, and the samples did not differ from each other on the first day of storage but B2, B3, and B4 of the first day show significant differences to B1 and B4 of the fifth day, along with B3 and B4 of the fifth day. On the issue of bread hardness, when comparing the values of a single sample between the first and the fifth day of storage, B1 and B4 differed statistically from the others, and on the fifth day of manufacture, there was an increase in the hardness value.

Table 6. Texture profile of the breads on the first and the fifth day of storage.

Bread	Resilience (%) $\bar{x} \pm sd$	Cohesiveness $\bar{x} \pm sd$	Springiness (%) $\bar{x} \pm sd$	Hardness (N) \bar{x} (qd)	Gummy (N) \bar{x} (qd)	Chewiness (J) \bar{x} (qd)
First day						
B1	33.58 \pm 3.43 ^{Aa}	0.69 \pm 0.04 ^{Aa}	81.35 \pm 5.02 ^{Aa}	1451.88 (533.89)	1036.77 (343.81)	843.54 (242.15)
B2	34.52 \pm 4.92 ^{Aa}	0.69 \pm 0.07 ^{Aa}	83.97 \pm 5.71 ^{Aa}	86.88 (49.77)	61.33 (29.69)	53.99 (25.09)
B3	31.17 \pm 3.22 ^{Aab}	0.65 \pm 0.02 ^{Aab}	82.00 \pm 3.04 ^{Aa}	100.88 (18.06)	64.90 (10.90)	51.61 (8.59)
B4	35.18 \pm 2.22 ^{Aa}	0.72 \pm 0.03 ^{Aa}	85.79 \pm 2.15 ^{Aa}	60.78 (405.91)	42.717 (284.98)	36.98 (237.97)
Fifth day						
B1	24.77 \pm 3.92 ^{Bbc}	0.56 \pm 0.04 ^{Bbc}	79.93 \pm 2.64 ^{Ba}	5392.63 (786.64)	2880.96 (572.38)	2258.95 (516.20)
B2	24.08 \pm 2.41 ^{Bbc}	0.56 \pm 0.04 ^{Bbc}	79.29 \pm 5.74 ^{Ba}	80.94 (1569.50)	44.75 (850.99)	33.77 (709.87)
B3	19.80 \pm 3.94 ^{Bc}	0.48 \pm 0.06 ^{Bc}	79.87 \pm 4.42 ^{Ba}	4641.25 (1168.35)	2035.03 (621.63)	1597.31 (565.38)
B4	21.66 \pm 2.43 ^{Bc}	0.52 \pm 0.03 ^{Bc}	80.61 \pm 3.20 ^{Ba}	6305.55 (840.85)	3183.83 (453.31)	2598.46 (410.55)

For parameters resilience, cohesiveness, and elasticity values followed by lowercase letters (^{a,b,c}) compare results between treatments for each day and uppercase letters (^{A,B,C}) compare results of the same treatment in the interval of the first and the fifth day.

The same letters do not differ significantly by the Tukey test ($p > 0.05$).

\bar{x} = mean; sd = standard deviation; \bar{x} = median of the values; qd = quartile deviation.

Table 7. Conover-Iman test for the variables gummy, chewiness, and hardness.

Sample	B1 _{1d}	B1 _{5d}	B2 _{1d}	B2 _{5d}	B3 _{1d}	B3 _{5d}	B4 _{1d}
Gummy							
B1 _{5d}	0.2960	–	–	–	–	–	–
B2 _{1d}	1.0000	0.0104*	–	–	–	–	–
B2 _{5d}	1.0000	0.0540	1.0000	–	–	–	–
B3 _{1d}	1.0000	0.0110*	1.0000	1.0000	–	–	–
B3 _{5d}	1.0000	1.0000	0.2960	1.0000	0.2984	–	–
B4 _{1d}	1.0000	0.0084*	1.0000	1.0000	1.0000	0.2549	–
B4 _{5d}	0.2752	1.0000	0.0094*	0.0493*	0.0104*	1.0000	0.0076*
Chewiness							
B1 _{5d}	0.4460	–	–	–	–	–	–
B2 _{1d}	1.0000	0.0220*	–	–	–	–	–
B2 _{5d}	1.0000	0.0890	1.0000	–	–	–	–
B3 _{1d}	1.0000	0.0170*	1.0000	1.0000	–	–	–
B3 _{5d}	1.0000	1.0000	0.4210	1.0000	0.3450	–	–
B4 _{1d}	1.0000	0.0170*	1.0000	1.0000	1.0000	0.3450	–
B4 _{5d}	0.3500	1.0000	0.0170*	0.0630	0.0120*	1.0000	0.0120*
Hardness							
B1 _{5d}	0.2708	–	–	–	–	–	–
B2 _{1d}	1.0000	0.0064*	–	–	–	–	–
B2 _{5d}	1.0000	0.0659	1.0000	–	–	–	–
B3 _{1d}	1.0000	0.0114*	1.0000	1.0000	–	–	–
B3 _{5d}	1.0000	1.0000	0.1559	1.0000	0.2708	–	–
B4 _{1d}	1.0000	0.0064*	1.0000	1.0000	1.0000	0.1559	–
B4 _{5d}	0.2708	1.0000	0.0064*	0.0659	0.0114*	1.0000	0.0064*

1_d = first day of storage; 5_d = fifth day of storage.

*Significant difference to the 95% confidence interval.

Breads formulated with sourdough did not show statistical variation for the texture profile after five days of storage, therefore, there was no decrease in quality, in the evaluated parameters, during the evaluated period, contrary to what occurred for bread made with baker's yeast, which showed greater hardness, chewability, and gumminess. Considering the texture profile and the microbiological evaluation, breads formulated with sourdough would potentially have a longer shelf life compared to the control treatment.

These results indicate a tendency to delay staling of bread by the use of sourdough. Production of organic acids by LAB may be related to delay staling bread due activity of amylases and wheat proteases - favoured by the drop of pH. The activity of these

enzymes promotes various changes in physicochemical characteristics of the starch and gluten network leading decrease in hardness and staling. For example, the low molecular weight resulting from starch hydrolysis promoted by amylases are not available for retrogradation. Moreover, those saccharides interfere with starch-protein interactions in the aging bread, which decreases firming (Arendt et al. 2007; Tebben et al. 2018).

The role of enzymes such as amylases and proteases is so much relevant in the quality of breads that they have been used as additives, mainly to extend the shelf life of the baked product (Dahiya et al. 2020). Moreover, considering the production of EPS by LAB can improve texture properties and delays the staling of bread (Torrieri et al. 2014), the EPS synthesis from these starters and the correlation with the profile texture should be further investigated. Although the mechanisms leading to staling and the anti-staling properties of some LAB have not been fully understood, there is sufficient evidence in the literature to confirm that sourdough fermentation delays staling of bread (Arora et al. 2021).

Conclusions

The use of type II sourdough, with selected lactic acid bacteria, led to the production of breads with improved nutritional, microbiological, and technological properties. The sourdough breads had a lower concentration of FODMAPs, greater volume, and better performance during storage when compared to the control bread produced only with baker's yeast. The inclusion of selected starter culture in the sourdough type II allowed the reduction of fructans above 90%, thereby producing low FODMAP bread suitable for IBS patients. In addition, sourdough breads presented a better performance in relation to texture and microbiological quality during the storage period.

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