An Acad Bras Cienc (2022) 94(Suppl. 4): e20220091 DOI 10.1590/0001-3765202220220091
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

MICROBIOLOGY

Evolution of the spontaneous sourdoughs microbiota prepared with organic or conventional whole wheat flours from South Brazil

LETÍCIA F.X. COSTA, CAROLINE ISABEL KOTHE, TIELA T. GRASSOTTI, RAQUEL P. GARSKE, BEATRIZ N. SANDOVAL, ANA PAULA M. VARELA, JANIRA PRICHULA, JEVERSON FRAZZON, MICHELE B. MANN, ROBERTA C.S. THYS & ANA PAULA G. FRAZZON

Abstract: The purpose of this study was to compare the composition and stability of bacteria and fungi communities during the propagation of sourdoughs prepared with organic or conventional whole wheat (*Triticum aestivum*) flours from South Brazil. Sourdoughs were prepared and samples were collected during different fermentation times (0 to 216 h). Total DNA of sourdough samples were extracted and the *16S rRNA* gene and Internal Transcribed Spacer region were sequenced by MiSeq-Illumina. A total of 43 and 56 OTUs were identified and defined as core taxa in the bacterial and fungal communities, respectively. The analysis revealed increases in the relative abundances of the lactic acid (*Pediococcus pentosaceus, Weissella hellenica* and *Limosilactobacillus pontis*) and acetic acid bacteria (*Gluconobacter frateurii* and *Acetobacter tropicalis*) during the sourdough propagation. The filaments fungi, *Alternaria tenuissima, Fusarium culmorum, Fusarium petersiae* and *Microdochium seminicola* remained more stable in organic than conventional during propagation cycles. After 216 h of fermentation, both sourdoughs were dominated by acid- and salt-tolerant yeast *Issatchenkia orientalis* (syn *Pichia kudriavzevii*, and *Candida glycerinogenes*). In conclusion, there were no significant differences in microbial communities among the sourdough samples. This study revealed that both flours contain autochthonous LAB, AAB, and yeasts with biotechnological applications in sourdough bread-making.

Key words: acetic acid bacteria, lactic acid bacteria, *Issatchenkia orientalis,* microbial communities, wheat flour, sourdough.

INTRODUCTION

Sourdough can be described as a type of dough containing a mixture of flour and water, spontaneously fermented by active microorganisms or can be reactivated (addition of flour and water), and resulting in an end product with a sour taste (De Vuyst & Neysens 2005, Gobbetti et al. 2014, Minervini et al. 2015). Based on the kind of technology applied for their production, as used in artisan and industrial processes, sourdoughs have been classified into three types. Type I, called traditional sourdough, is most the widely used in artisan bakeries and it is characterized by daily propagation to remain the microorganisms in active metabolic state. Type II sourdough is used mostly in industrial processes and require the addition of baker's yeast (*Saccharomyces cerevisiae*) and/or specific bacteria (as *Lactobacillus* spp.), as a

staters cultures, into a flour-water mixture. This sourdough is fermented for a long period (up to 5 days) within a temperature range from 30 °C to 50 °C and without feedings, which reduces the microbial activity. Type III is produced by dehydrating the stabilized form of type II sourdough and essentially used at the industrial level. It is used as acidifier supplements and aroma carriers during bread making. This sourdough is dominated by drying-resistant lactic acid bacteria (LAB), such as *Pediococcus pentosaceus*, *Lactobacillus plantarum*, and *Lactobacillus brevis* (De Vuyst & Neysens 2005, Yazar & Tavman 2012, Calvert et al. 2021).

Researchers have shown that the diversity of sourdough microbiota can be modulated by time, temperature, and/or types of flour (Gänzle 2014, Pontonio et al. 2016). Flour is the main source of microorganisms responsible for the spontaneous sourdough fermentation (Gobbetti et al. 2014, Reese et al. 2020). The sourdough starters microbial communities showed to have a high relative abundance of LAB belonging to the family Lactobacillaceae, yeasts (species of diverse genera within the order Saccharomycetales) and acetic acid bacteria (AAB) such as *Acetobacter* species (De Vuyst et al. 2016, Reese et al. 2020, Landis et al. 2021). These microorganisms are associated with acidification, ethanol, and carbon dioxide formation, that are desirable characteristics of good quality bread (Gobbetti et al. 2014, Rizzello et al. 2015, Reese et al. 2020).

Whole wheat flour is one of the healthiest options in sourdough production since it contains greater amounts of antioxidants, and minerals (Vaher et al. 2010, Katina et al. 2012, De Angelis et al. 2018). The use of organic whole wheat flour in baking has gained prominence in the production. In the last year, the green concept has contributed to the increase in the production and consumption of organic food worldwide (Popa et al. 2019, Simonetti et al. 2019). In addition, there has been growing interest in studies to evaluate the nutritional content of wheat flours from organic production system. The results showed that organic samples had high-quality proteins and microelements contents (Vrček et al. 2014). Contrariwise, another study did not observe significant differences in humidity, protein and gluten between organic and conventional flours (Draghici et al. 2011). Lazo-Vélez et al. (2021) pointed out that organic wheat flour usually contains low gluten, poor dough rheological properties, and high diastatic activity, which produce a bread with low quality and volume, mainly due to the weaker gluten.

To date, few studies have investigated the sourdoughs microbiota composition and stability using organic or conventional whole wheat flours, and these studies were carried out mainly in Italy (Minervini et al. 2015, Rizzello et al. 2015, Pontonio et al. 2016, 2021). So, in this context, our study aimed to compare the composition and stability of bacteria and fungi communities during the propagation of sourdoughs prepared with organic or conventional whole wheat (*Triticum aestivum*) flours from South Brazil.

MATERIALS AND METHODS

Whole wheat flours

Commercially available conventional (Panfácil, Canoas, Brazil) and organic (Ecobio, Coronel Bicaco, Brazil) whole wheat flours (*T. aestivum*) from regional production were purchased in Porto Alegre, South Brazil. Nutritional composition described in the flour labels was presented in Supplementary Material - Table SI.

Sourdough preparation and propagation

The sourdoughs were prepared according to Menezes et al. 2019, with modifications (Fig. 1). Sourdough was prepared by mixing organic

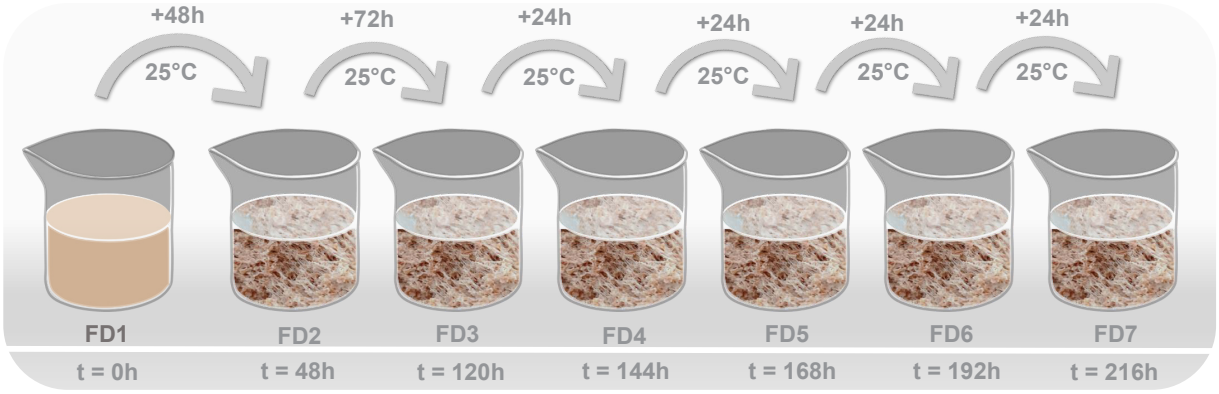

Figure 1. Backslopping to produce fermented dough from organic and conventional whole wheat flours. FD1, FD2, FD3, FD4, FD5, FD6, and FD7: fermented dough 0 h, 48 h, 120 h, 144 h, 168 h, 192 h, and 216 h of fermentation, respectively.

(O) or conventional (C) whole wheat flours and sterile tap water at a 1:1 (w/v) ratio. These non-fermented doughs (FD) (FD1; 0 h) were incubated at 25 °C for 48 h. Subsequently, these backslopping were used to propagate the FD2 (48 h) through FD7 (216 h) - by mixing the previously fermented dough, sterile tap water and flour at a ratio of 1:2:2 (w/v/w). All the FDs were also incubated at 25 °C.

pH analysis and total titratable acidity (TTA)

Fermented dough samples from FD1 to FD7 were collected, and 10 g from each sample were diluted in 100 mL of distillate water to determine the pH and total titratable acidity (TTA). The pH was determined by using a digital pH meter (HI 2221, Hanna® Instruments, Barueri, Brazil) at room temperature. The TTA was expressed in NaOH 0.1 mol / L, in mL, required to pH 8.5 according to standard analytical standards of Adolfo Lutz Institute (IAL 2005). Measurements were made in triplicate.

We used Fischer's exact test and a chi-square test, where $p < 0.05$ was found to be a significant difference factor between the samples evaluated, and SAS On-Demand for Academics Software (SAS Institute Inc., United States) for statistical analyses.

Microbial sequence by high throughput sequencing

High-throughput sequencing was used to assess the structure and composition of microbiota communities in organic (O) and conventional (C) whole wheat flours, and FD1, FD3, FD5 and FD7 sourdoughs prepared with organic or conventional whole wheat flours. Total DNA from the samples was extracted using E.Z.N.A. kit (Bio-Tek) according to the manufacturer's instructions. The DNA concentration was determined using the Qubit® 3.0 fluorometer (Thermo Fischer Scientific), and its quality was verified using the NanoDrop ND-1000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Three independent replicates of each sample were used for DNA extraction and amplicon generation.

To characterize the bacterial community, amplification of the variable region V4 region of the *16S rRNA* was carried out using the primers described by Caporaso et al. 2011 and Endres et al. 2021. PCR reaction was performed in a total volume of 50 µL containing 1x buffer, 0.2 mM dNTPs, 0.2 µM of each primer, 1.5 mM of $MgCl_2$, 2U of Platinum *Taq* DNA polymerase, 10 ng DNA, and water to complete the volume. The

PCR was carried out in Biorad MyCycler Thermal Cycle under the following conditions: 94 °C for 3 minutes, followed by 30 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds, and a final extension of 72 °C for 5 minutes.

To characterize the fungal community, the ITS (*18S rRNA*) regions region was amplified using primers previously described by White et al. 1990 and Gardes & Bruns 1993. The PCR reactions were performed in a total volume of 50 µL containing 1x buffer, 0.2 mM dNTPs, 0.16 µM of each primer, 2.5 mM $MgCl_2$, 2U of with Platinum *Taq* DNA polymerase (Invitrogen, California, USA), 10 ng DNA, and water to complete the volume. The PCR was carried out in Biorad MyCycler Thermal Cycle under the following conditions: 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 45 seconds, 56 °C for 45 seconds, and 72 °C for 1 minutes, and a final extension of 72 °C for 10 minutes.

The PCR products were purified using Agencourt AMPure XP (Beckman Coulter, Indianapolis, IN) according to the manufacturer's protocol. The indexes were added to the DNA libraries following the manufacturer's instructions (Illumina Inc., San Diego, California, USA). The sequencing was performed in the Illumina MiSeq instrument using the MiSeq Reagent Kit v2 (500-cycles). Sequences generated were submitted to the NCBI database under accession number PRJNA632575.

Microbiota community analysis

The raw data of the sequencing were evaluated for their quality by FastQC (Andrews 2018), and the sequences were analyzed using FROGS (Find Rapidly OTUs with Galaxy Solution) pipeline (Escudié et al. 2018) to obtain the Operational Taxonomic Units (OTUs). The sequences were filtered by 340-500 bp and then grouped into OTUs using SWARM (Mahé et al. 2015) with the distance parameter "d = 3". Chimeras were removed by VSEARCH (Rognes et al. 2016) and OTUs with at least 0.1% of reads were retained. The OTUs were evaluated using the SILVA 132 SSU database for bacteria determination (Quast et al. 2013) and UNITE 8.2 for fungi (UNITE 2021).

The package "phyloseq" (v1.30.0) of the software R Studio v.3.6.1 was used to explore the microbial community from metagenomic data (McMurdie & Holmes 2013). The relative abundance of genera present in the samples was plotted to relieve the "plot_composition" function. A hierarchical grouping based on the Bray-Curtis distance was plotted using the ward.D2 method and observed with the "hclust" function. The richness was estimated with "plot_richness" and plotted with the boxplot function using the indices "Chao1", "se.chao1", "Simpson", and "InvSimpson" (Wickham et al. 2010). A principal coordinate analysis (PCoA) plot was used to generate beta-diversity and measure the degree of differentiation among the samples. All associations with $p > 0.05$ were considered insignificant.

RESULTS AND DISCUSSION

pH and TTA in sourdough prepared with organic and conventional whole soft wheat flour during propagation cycles

The result revealed significant ($p < 0.05$) differences in the pH and TTA values between sourdoughs prepared with organic and conventional whole wheat flours and among the propagation cycles (Table I). The pH and TTA did vary during the fermentation propagation. The pH of sourdoughs prepared with organic flour ranged from 6.43 to 4.30, while the pH of conventional flour ranged from 6.57 to 4.22 in. TTA in the begging of the fermentation (FD1) were 3.68 and 3.3 (ml 0.1 N NaOH/ l00 g of dough) and in the end (FD7) were 19.22 and

Table I. The pH and total titratable acidity (TTA) values on fermented doughs prepared with organic or conventional whole wheat flours.

Samples	Time	pH		TTA (mL NaOH 0.1 M/ 100g)	
		Organic	Conventional	Organic	Conventional
FD1	0 h	6.43 ± 0.01 ^{aB}	6.57 ± 0.02 ^{aA}	3.68 ± 0.12 ^{fA}	3.60 ± 0.02 ^{fA}
FD2	48 h	4.72 ± 0.00 ^{bB}	4.93 ± 0.04 ^{bA}	13.11 ± 0.11 ^{eB}	19.35 ± 0.59 ^{eA}
FD3	120 h	4.34 ± 0.02 ^{deB}	4.42 ± 0.01 ^{dA}	22.51 ± 0.02 ^{aB}	24.93 ± 0.09 ^{aA}
FD4	144 h	4.49 ± 0.04 ^{cA}	4.49 ± 0.02 ^{cdA}	16.74 ± 0.17 ^{dB}	21.67 ± 0.10 ^{dA}
FD5	168 h	4.36 ± 0.02 ^{dA}	4.41 ± 0.02 ^{dB}	18.62 ± 0.32 ^{bB}	23.62 ± 0.05 ^{bA}
FD6	192 h	4.27 ± 0.03 ^{fB}	4.53 ± 0.02 ^{dA}	17.68 ± 0.33 ^{cB}	23.41 ± 0.00 ^{bA}
FD7	216 h	4.30 ± 0.01 ^{efA}	4.22 ± 0.05 ^{cB}	19.22 ± 0.37 ^{bB}	22.67 ± 0.09 ^{cA}

FD: Fermented dough; TTA: total titratable acidity. Same lowercase letters in the same columns means there is no significant difference ($p > 0.05$) between fermented dough samples. Same uppercase letters in the same line means there is no significant difference ($p > 0.05$) between organic and conventional samples.

22.67, in sourdoughs made from organic and conventional flours, respectively. The pH and TTA values in all sourdoughs are consistent with previous studies (Aplevicz et al. 2013, Hadaegh et al. 2017).

Organic acids production is an essential step in sourdough fermentation, as it provides an ideal growth environment for a specific group of microorganisms, including LAB, that produces lactic and acetic acid, within the pH range from 5.0 to 3.5 (De Vuyst et al. 2009, 2014, Liu et al. 2018, Oshiro et al. 2020, Demirkesen-Bicak et al. 2021). Consequently, the acidification resulting from microbial metabolism modulates the activity of cereal enzymes, such as proteases and amylases (Fraberger et al. 2020, Voidarou et al. 2021). By combining the information about organic acid production, it becomes possible to assess the quality of sourdough and thus maintain a constant quality in the final product.

Bacterial community structure in sourdoughs prepared with organic or conventional flours during propagation cycles

A total of 576,557 high-quality reads were obtained from the sourdoughs and flours samples. The DNA sequences were grouped into 43 OTUs with relative abundances greater than 0.1%. Most of the OTUs belonged to the Firmicutes phylum ($\overline{x}$= 43.80%), which were followed by Proteobacteria ($\overline{x}$= 0.71%) and Actinobacteria ($\overline{x}$ = 0.029%). The distribution of phyla in the FD samples was similar in both types of whole wheat flours (Supplementary Material - Figure S1a). Firmicutes was detected after 120 h of fermentation (FD3) in sourdoughs prepared with organic or conventional flours and remained stable throughout the end of the propagation time (216 h; FD7). The occurrence of Firmicutes could be associated with the increase of the TTA in the sourdoughs after 120 h of fermentation (Table I), since some species of this phylum are acid tolerant, such as *Latilactobacillus, Levilactobacillus* and *Pediococcus* (De Vuyst et al. 2014, Minervini et al. 2015, Comasio et al. 2020). This association has already been carried

out in other studies (De Vuyst et al. 2009, 2014, Liu et al. 2018, Demirkesen-Bicak et al. 2021). The presence of *Latilactobacillus, Levilactobacillus* and *Pediococcus* is generally related to very low pH values. However, Oshiro et al. 2020 also observed a predominance of LAB in sourdough with not too low pH (6.7, 5.5, and 4.5), as found in the present study. Flour intrinsic parameters can explain these differences since they also modulate the establishment of sourdough microbiota and, consequently, the acid organic formation.

Proteobacteria and Actinobacteria were observed in all evaluated samples. The bacterial dynamic in sourdoughs prepared with organic or conventional whole wheat flours is consistent with the results from previous studies, that investigate the microbial diversity in sourdough made with organic (Rizzello et al. 2015, Pontonio et al. 2021) or conventional wheat flours in Italy (Ercolini et al. 2013, Minervini et al. 2015).

A total of 11 families showed a relative abundance of > 0.1% (Figure S1b). Enterobacteriaceae was the dominant family in the flours (O and C) and FD1samples. However, after 120 h of fermentation (FD3) the dominant family in both sourdoughs was Lactobacillaceae ($\bar{x}$= 78.3%). Acetobacteraceae was also observed in the FD5 and FD7 samples ($\bar{x}$= 10.9%).

Thirteen genera were detected with a relative abundance of ≥ 0.1% (Fig. 2a). Sourdoughs prepared with organic whole wheat flour (FD3 to FD7) have the lowest richness and evenness when compared to conventional whole wheat flour. *Pediococcus* sp., *Latilactobacillus* sp., *Limosilactobacillus* sp., *Levilactobacillus* sp. *Companilactobacillus* sp. *Acetobacter* sp. and *Gluconobacter* sp., were predominated in FD3, FD5 and FD7sourdoughs prepared with conventional flour. At the same time, *Pediococcus* sp. was dominant in all sourdoughs prepared with organic flour, followed by *Levilactobacillus* sp. and *Gluconobacter* sp. This lower species richness could be related to nutritional proprieties of organic flour, since nutrients are necessary for microbial growth. Some studies have been showing that organic flours had lower protein, calcium, manganese and iron contents when compared to conventional flours (Vrček et al. 2014).

Pediococcus pentosaceus and *Gluconobacter frateurii* were the main LAB and AAB species, respectively, found in FD7 sourdough prepared with organic whole wheat flour, while *Levilactobacillus brevis, Latilactobacillus sakei, Limosilactobacillus pontis* (LAB species) and *Acetobacter tropicalis* (AAB species) were dominant in FD5 and FD7 sourdoughs prepared with conventional whole wheat flour (Fig. 2b). The occurrence of LAB and AAB species in our samples corroborates with previous results, that demonstrated the higher abundance of these microorganisms in sourdough microbiota (De Vuyst et al. 2016, Landis et al. 2021). These microorganisms are also associated with acidification and flavor formation, which are both related to carbohydrate metabolism (De Vuyst et al. 2016, Van Kerrebroeck et al. 2017, Siepmann et al. 2019). Furthermore, the occurrence of AAB and LAB in the samples can lead to the lowest pH and highest TTA observed in sourdough samples (Table I).

Alpha-diversity analysis showed no significant differences (p > 0.05) between bacterial communities in sourdoughs prepared with organic or conventional flours (Fig. 2c). In addition, no significant difference in beta-diversity was observed between sourdoughs made with organic or conventional flours (p > 0.05) (Fig. 2d).

The higher abundance of mitochondrial and chloroplast DNA in pure flours (O and C) and FD1 samples, have already determinate in previous studies (Ercolini et al. 2013, Minervini et al. 2015).

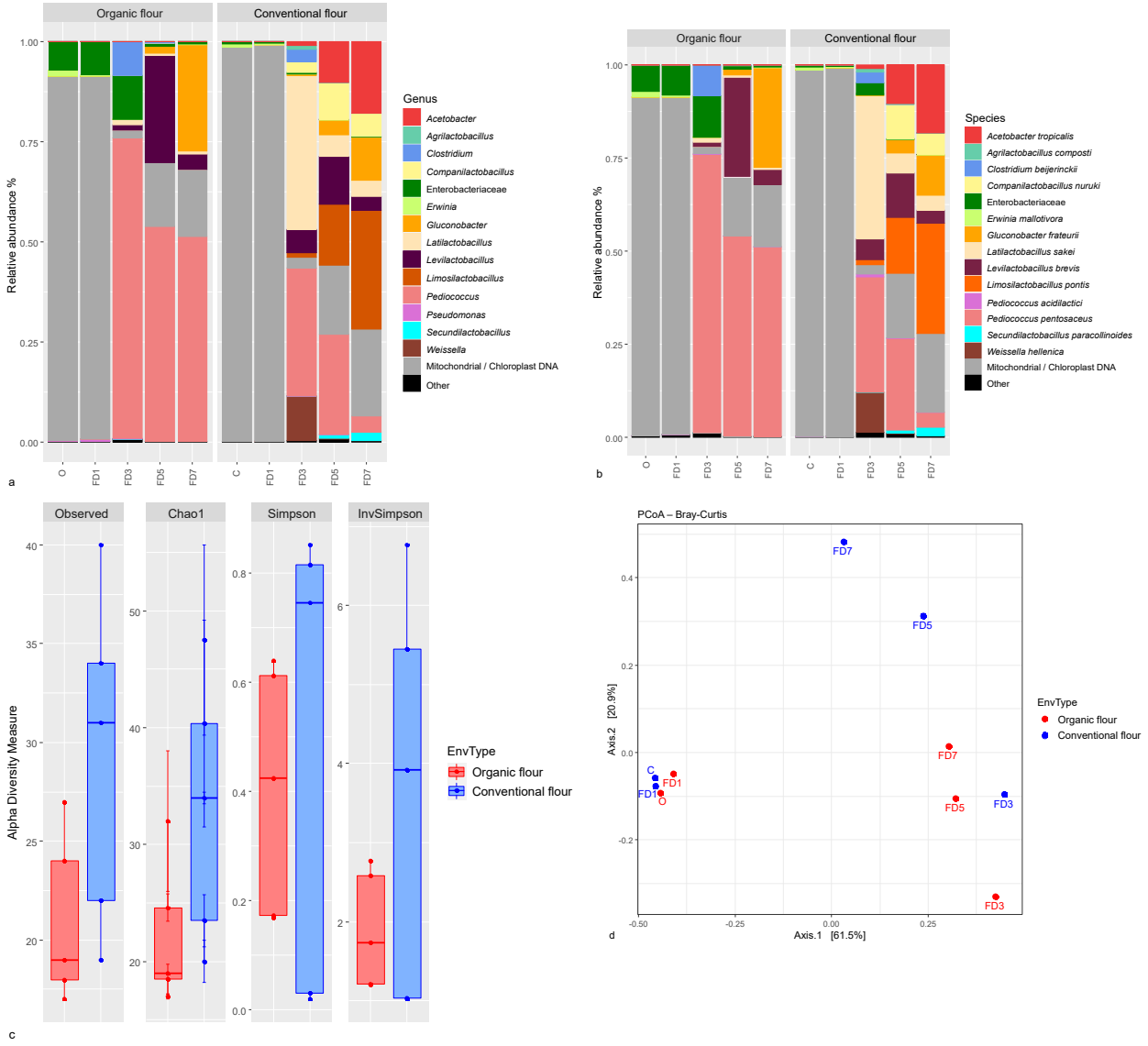

Figure 2. Bacterial genus (a), species (b), alpha-diversity (c) and beta-diversity for PCoA (d) based on Bray-Curtis evaluation for organic and conventional flours and sourdough samples during leavening. O: organic whole wheat flour; C: conventional whole wheat flour; FD1 (0 h), FD3 (120 h), FD5 (168 h), and FD7 (216 h).

Wheat mitochondria and chloroplasts genomes contain conserved DNA regions that are targeted by oligonucleotides (Hanshew et al. 2013, Yang et al. 2014).

Fungal community structure in sourdoughs made with organic or conventional flours during propagation cycles

A total of 262,452 high-quality readings from sequencing. The DNA sequences from the ITS region were grouped into 56 OTUs, which comprised three phyla (Figure S2a). Ascomycota was dominant phylum in all samples ($\bar{x}$= 99.3%), followed by Basidiomycota ($\bar{x}$ = 0.6%) and Mucoromycota ($\bar{x}$= 0.1%). This result is consistent with previous studies that shown Ascomycota phylum is a natural member of flour fungal composition (Minervini et al. 2015, Urien et al. 2019). The distribution of fungal phyla found in

sourdoughs made with organic and conventional whole wheat flours were similar (Figure S2a).

We detected a total of 22 families with a relative abundance of > 0.1%, including Saccharomycetaceae ($\bar{x}$ =57.2%), Metschnikowiaceae ($\bar{x}$= 14.9%), Pleosporaceae ($\bar{x}$=10.0%), Nectriaceae ($\bar{x}$=8.7%), and Microdochiaceae ($\bar{x}$= 4.7%) (Figure S2b). Differences in the family's occurrence were observed in sourdough samples prepared with organic or conventional flours. Saccharomycetaceae was the dominant at the beginning of fermentation (FD3) in conventional flour, and in the end of fermentation (FD7) in organic flour.

Differences at the genera level were also detected in sourdough samples. The FD1, FD3 and FD5 sourdoughs prepared with organic flour were composed mainly of filamentous fungi, such as *Alternaria* sp., *Fusarium* sp. and *Microdochium* sp., while FD3 and FD5 prepared with conventional flour were dominated by yeast fungi, such as *Issatchenkia* sp. and *Diutina* sp. (Fig. 3a). The filamentous fungal community in sourdoughs fermentation (FD1, FD3 and FD5 propagation) made with organic flour were more stable than conventional flour. This might have happened because lesser-toxic and natural pesticides are used in organic farming.

Both FD7 sourdoughs were dominated by acid- and salt-tolerant yeast *Issatchenkia orientalis* (syn *Pichia kudriavzevii*, and *Candida glycerinogenes*) (Fig. 3b). This yeast has been isolated from a variety of environments, including sourdough around the world, and pointed out to be used in the development of starter cultures to produce better quality fermented products from masau fruit (Nyanga et al. 2007, De Vuyst et al. 2014, Matsushika et al. 2016). The six yeast species that are often encountered in stable sourdoughs are: *S. cerevisiae*, *Kazachstania exigua, Pichia kudriavzevii, Torulaspora delbrueckii, Wickerhamomyces anomalus* and *Hansenula anomala* (De Vuyst et al. 2014, 2016, Landis et al. 2021).

Finally, for fungal communities, there were no significant differences in alpha- and beta-diversity between sourdoughs prepared with organic or conventional flours (p > 0.05) (Fig. 3c and 3d).

Microbial community dynamic in sourdoughs with organic or conventional flours during fermentation

We also evaluated the Bray-Curtis metric to beta-diversity and organized it as clusters. The bacterial communities during the propagation of sourdoughs were not related to the initial microbiota (O or C pure flours and FD1) (Figure S3a). Regarding fungi, the pure flours (O), non-fermented doughs (FD1), and propagated sourdough samples from organic flour (FD3 and FD5) were segmented in one cluster, while sourdough samples from conventional flour (FD3, FD5, and FD7) and organic flour (FD7) were in another cluster (Figure S3b).

The dynamics of the bacterial communities were altered according to the leavening time. For example, the frequency of Enterobacteriaceae and *Erwinia* sp. observed in the organic (O) and conventional (C) flour and FD1 s decreased to less than 0.1% after 168 h of fermentation (FD5). The reduction of enterobacteria's might be associated with the increase of LAB and AAB during fermentation time since these bacteria are able to produce organic acids from carbohydrates metabolism (Comasio et al. 2020). The AAB group is responsible for oxidative fermentation, which occurs when carbohydrates, alcohols, and alcoholic sugar are converted into organic acids, aldehydes, and ketones (Halstead et al. 2015, Lynch et al. 2019). Acidity inhibits most microbial growth - including spoilage microorganisms – and it is used frequently for

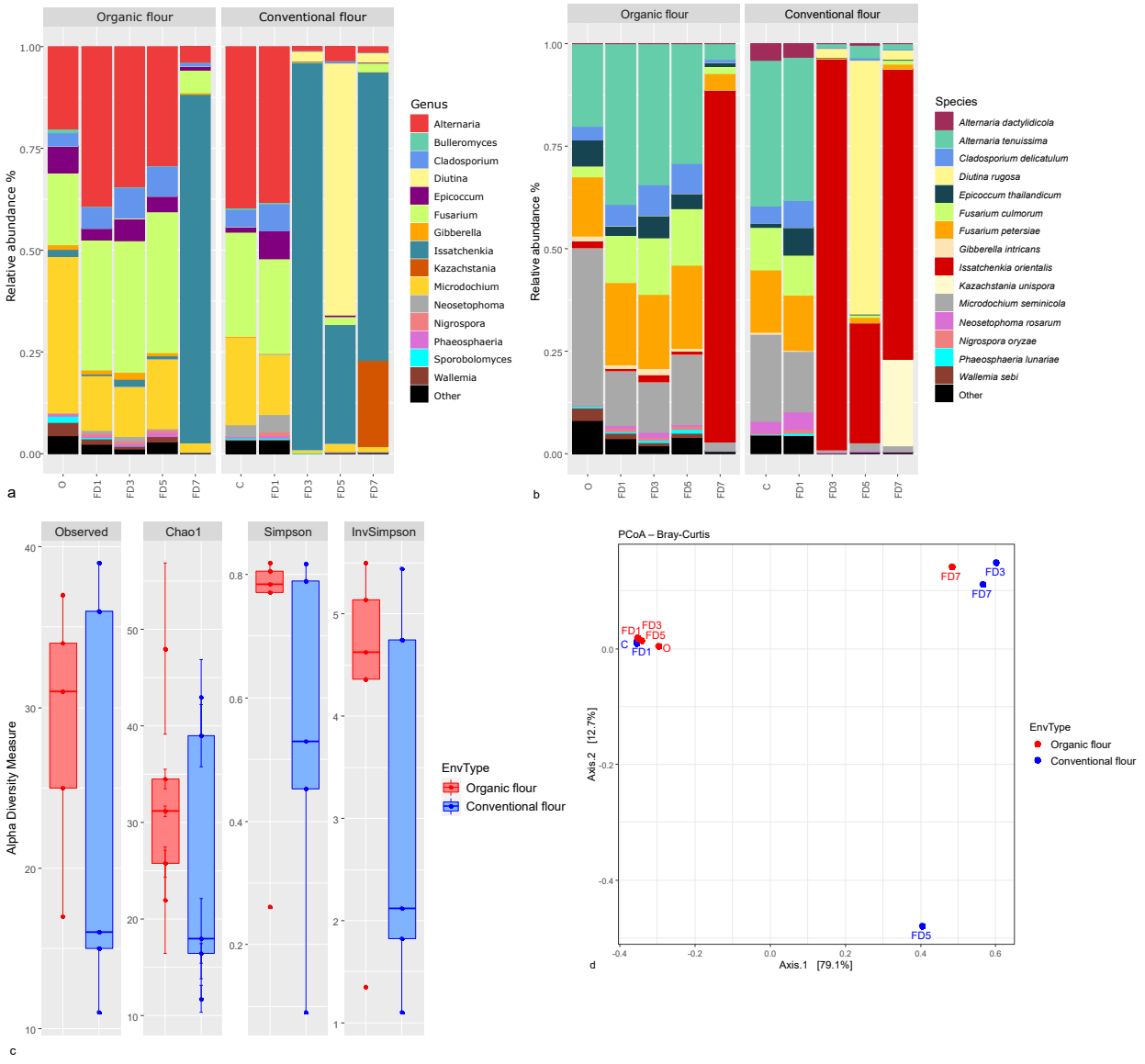

Figure 3. Fungal genus (a), species (b), alpha-diversity (c) and beta-diversity for PCoA (d) based on Bray-Curtis evaluation for organic and conventional flours and sourdough samples during leavening. O: organic whole wheat flour; C: conventional whole wheat flour; FD1 (0 h), FD3 (120 h), FD5 (168 h), and FD7 (216 h).

food preservation (Horackova et al. 2018, Liu et al. 2018, Bartkiene et al. 2020).

The fungal dynamic was related to whole wheat flours used and the variation from the backslopping steps (due to the repositioning of the flour). The filaments fungi, *Alternaria tenuissima, Fusarium culmorum, Fusarium petersiae* and *Microdochium seminicola* were stable in organic whole wheat (from O until FD5) (Fig. 3b). The reduction of fungal species during the fermentation process probably occurred due to the formation of secondary metabolites generated during the metabolism of the active microbiota in the sourdough (Azevedo et al. 2020). These metabolites associated with organic acidy production, can improve the shelf life of sourdough bread and reduce product spoilage (Quattrini et al. 2019). On the other hand, *I. orientalis* occurred in sourdough prepared with conventional whole wheat flour at 120 h

of fermentation (FD3) and was dominant until the end of the fermentation (FD7). Furthermore, *Diutina rugosa* and *Kazachstania unispora* were co-dominance with *I. orientalis* at 168 h (FD5) and 216 h of fermentation (FD7), respectively, in sourdough made with conventional flour. The abundance of yeasts is probably due to changes of pH, enzyme activity, substrate availability, temperature, microbial interactions, and foreign compounds (xenobiotics) at the end of the fermentation process (De Vuyst et al. 2014, see also the review of Lau et al. 2021). Previous research has also reported an increase in yeasts abundance after 72 h of fermentation (Comasio et al. 2020).

The microbial composition variation during the propagation cycles was associated with the acidic production (Table I) (Fig. 4). The acidity keeps out pathogenic microorganisms and favors the growth of yeasts, and some acid-tolerant bacteria, such LAB and AAB (De Vuyst et al. 2014). Our findings corroborate those of Ripari et al. 2016, who reported microbial composition variation over time, with LAB and yeasts becoming the most prevalent after four fermentation steps. The LAB and AAB have been associated with acidification and yeasts and some species of LAB to leavening.

In conclusion, the comparison of microbial communities during the propagation of sourdoughs prepared with organic or conventional whole wheat flours showed no differences. Although most investigations agree that sourdough bread prepared with organic flour yields has been commonly used, this study indicates that conventional wheat flour is also a good alternative to sourdough bread, mainly due to the absence of significant differences between the diversity of microbial communities of the flours. This study also demonstrated that

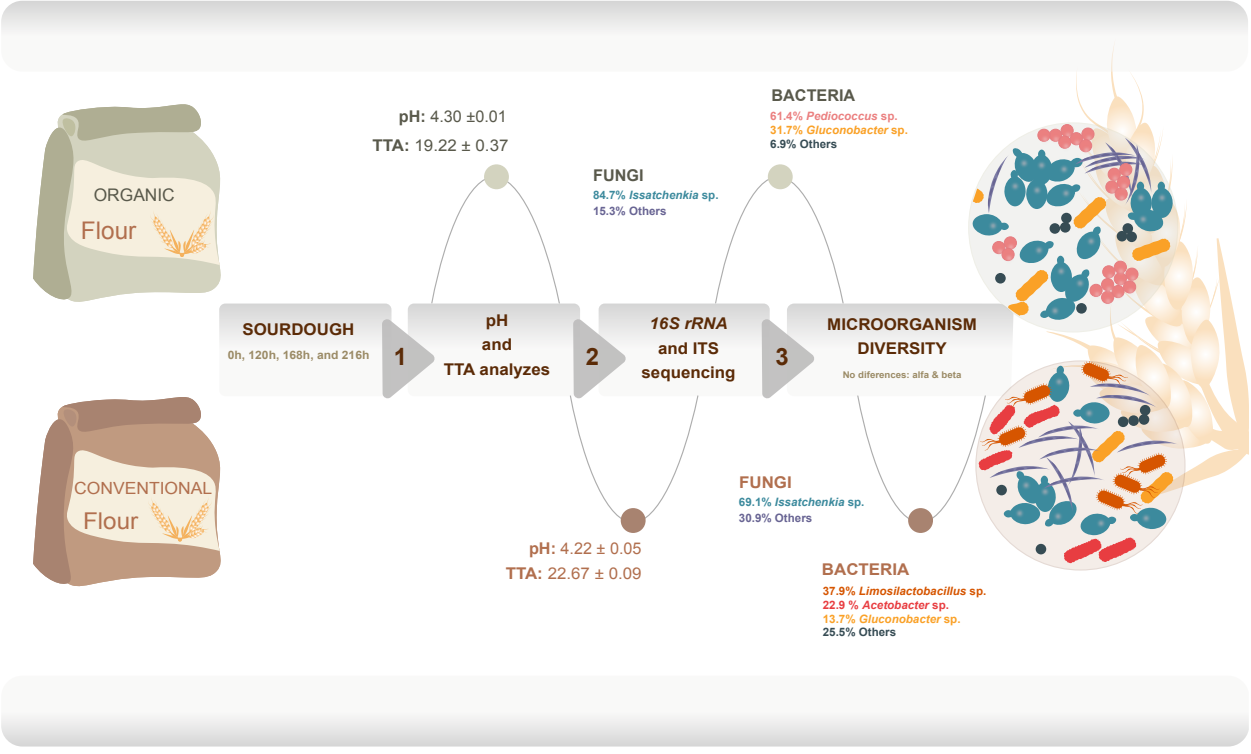

Figure 4. Scheme showing the main results obtained in spontaneous sourdoughs prepared with organic or conventional whole wheat flours from South Brazil.

sourdoughs prepared with organic flour showed more microbial stability than conventional whole wheat flour. This stability may be associated with the use of natural (or non-synthetic) pesticides in organic wheat crops. In addition, this study revealed that both flours contain autochthonous LAB, AAB, and yeasts with biotechnological applications in sourdough bread-making. Therefore, to our knowledge, this study provides, for the first time, information about microbial communities in sourdoughs prepared with organic or conventional whole wheat flour from South Brazil.

Acknowledgments

This work was performed within the framework of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) #302574/2017-0, #407886/2018-4 and #305495/2018-6#.

REFERENCES

ANDREWS S. 2018. FastQC: A Quality Control tool for High Throughput Sequence Data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed March 18, 2020).

APLEVICZ KS, OGLIARI PJ & SANT'ANNA ES. 2013. Influence of fermentation time on characteristics of sourdough bread. Braz J Pharm Sci 49: 233-239. https://doi.org/10.1590/S1984-82502013000200005.

AZEVEDO POS ET AL. 2020. Antibacterial and antifungal activity of crude and freeze-dried bacteriocin-like inhibitory substance produced by *Pediococcus pentosaceus*. Sci Rep 10: 12291. https://doi.org/10.1038/s41598-020-68922-2.

BARTKIENE E ET AL. 2020. Lactic acid bacteria isolation from spontaneous sourdough and their characterization including antimicrobial and antifungal properties evaluation. Microorganisms 8: 64. https://doi.org/10.3390/microorganisms8010064.

CALVERT MD, MADDEN AA, NICHOLS LM, HADDAD NM, LAHNE J, DUNN RR & MCKENNEY EA. 2021. A review of sourdough starters: ecology, practices, and sensory quality with applications for baking and recommendations for future research. PeerJ 9: e11389. https://doi.org/10.7717/peerj.11389. eCollection 2021.

CAPORASO JG, LAUBER CL, WALTERS WA, BERG-LYONS D, LOZUPONE CA, TURNBAUGH PJ, FIERER N & KNIGHT R. 2011. Global patterns of 16S rRNA diversity at a depth of a millions of sequences per sample. Proc Natl Acad Sci USA 108: 4516-4522. https://doi.org/10.1073/pnas.1000080107.

COMASIO A, VERCE M, VAN KERREBROECK S & DE VUYST L. 2020. Diverse microbial composition of sourdoughs from different origins. Front Microbiol 11. https://doi.org/10.3389/fmicb.2020.01212.

DE ANGELIS M, MINERVINI F, SIRAGUSA S, RIZZELLO CG & GOBBETTI M. 2018. Wholemeal wheat flours drive the microbiome and functional features of wheat sourdoughs. Int J Food Microbiol 302: 35-46. https://doi.org/10.1016/j.ijfoodmicro.2018.08.009.

DE VUYST L, HARTH H, VAN KERREBROECK S & LEROY F. 2016. Yeast diversity of sourdoughs and associated metabolic properties and functionalities. Int J Food Microbiol 239: 26-34. https://doi.org/10.1016/j.ijfoodmicro.2016.07.018.

DE VUYST L & NEYSENS P. 2005. The sourdough microflora: biodiversity and metabolic interactions. Trends Food Sci Technol 16: 43-56. http://doi.org/10.1016/j.tifs.2004.02.012.

DE VUYST L, VAN KERREBROECK S, HARTH H, HUYS G, DANIEL HM & WECKX S. 2014. Microbial ecology of sourdough fermentations: diverse or uniform? Food Microbiol 37: 11-29. https://doi.org/10.1016/j.fm.2013.06.002.

DE VUYST L, VRANCKEN G, RAVYTS F, RIMAUX T & WECKX S. 2009. Biodiversity, ecological determinants and metabolic exploitation of sourdough microbiota. Food Microbiol 26: 666-675. https://doi.org/10.1016/j.fm.2009.07.012.

DEMIRKESEN-BICAK H, ARICI M, YAMAN M, KARASU S & SAGDIC O. 2021. Effect of different fermentation condition on estimated glycemic index, in vitro starch digestibility, and textural and sensory properties of sourdough bread. Foods 1: 1-13. https://doi.org/10.3390/foods10030514.

DRAGHICI M, NICULITA P, POPA M & DUTA D. 2011. Organic wheat grains and flour quality versus conventional ones – consumer versus industry expectation. Rom Biotechnol Lett 16: 6572-6579.

ENDRES CM, CASTRO IMS, TREVISOL LD, SEVERO JM, MANN MB, VARELA APM, FRAZZON APG, MEYER FQ & FRAZZON J. 2021. Molecular characterization of the bacterial communities present in sheep's milk and cheese produced in South Brazilian Region via *16S rRNA* gene metabarcoding sequencing. LWT Food Sci Technol 147: 111579. https://doi.org/10.1016/j.lwt.2021.111579.

ERCOLINI D, PONTONIO E, DE FILIPPIS F, MINERVINI F, STORIA AL, GOBBETTI M & DI CAGNO R. 2013. Microbial ecology dynamics during rye and wheat sourdough preparation. App Environ Microbiol 79: 7827-7836. https://doi.org/10.1128/AEM.02955-13.

ESCUDIÉ F ET AL. 2018. FROGS: Find, rapidly, OTUs with Galaxy solution. Bioinformatics 34: 1287-1294. https://doi.org/10.1093/bioinformatics/btx791.

FRABERGER V, AMMER C & DOMIG KJ. 2020. Functional properties and sustainability improvement of sourdough bread by lactic acid bacteria. Microorganisms 8: 1895. https://doi.org/10.3390/microorganisms8121895.

GÄNZLE MG. 2014. Enzymatic and bacterial conversions during sourdough fermentation. Food Microbiol 37: 2-10. https://doi.org/10.1016/j.fm.2013.04.007.

GARDES M & BRUNS TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Mol Ecol 2: 113-118. https://doi.org/10.1111/j.1365-294x.1993.tb00005.x.

GOBBETTI M, RIZZELLO CG, DI CAGNO R & DE ANGELIS M. 2014. How the sourdough may affect the functional features of leavened baked goods. Food Microbiol 37: 30-40. https://doi.org/10.1016/j.fm.2013.04.012.

HADAEGH H, ARDABILI SMS, EBRAHIMI MT, CHAMANI M & NEZHAD RA. 2017. The impact of different lactic acid bacteria sourdoughs on the quality characteristics of toast bread. J Food Qual 2017: 7825203. https://doi.org/10.1155/2017/7825203.

HALSTEAD FD, RAUF M, MOIEMEN NS, BAMFORD A, WEARN CM, FRAISE AP, LUND PA, OPPENHEIM BA & WEBBER MA. 2015. The antibacterial activity of acetic acid against biofilm-producing pathogens of relevance to burns patients. PLoS ONE 10: e0136190. https://doi.org/10.1371/journal.pone.0136190.

HANSHEW AS, MASON CJ, RAFFA KF & CURRIE CR. 2013. Minimization of chloroplast contamination in *16S rRNA* gene pyrosequencing of insect herbivore bacterial communities. J Microbiol Methods 95: 149-155. https://doi.org/10.1016/j.mimet.2013.08.007.

HORACKOVA S, NOVAKOVA T, SLUKOVA M, BIALASOVA K, KUMHEROVA M & PLOCKOVA M. 2018. Antifungal activity of selected lactobacilli intended for sourdough production. App Food Biotechnol 5: 213-220. https://doi.org/10.22037/afb.v5i4.22173.

IAL - ADOLFO LUTZ INSTITUTE. 2005. Métodos físico-químicos para análise de alimentos, 4th ed. Instituto Adolfo Lutz, Brasília, 1000 p.

KATINA K, JUVONEN R, LAITILA A, FLANDER L, NORDLUND E, KARILUOTO S, PIIRONEN V & POUTANEN K. 2012. Fermented wheat bran as a functional ingredient in baking. Cereal Chemistry J 89: 126-134. https://doi.org/10.1094/cchem-08-11-0106.

LANDIS EA ET AL. 2021. The diversity and function of sourdough starter microbiomes. ELife 10: e61644. https://doi.org/10.7554/eLife.61644.

LAU SW, CHONG AQ, CHIN NL, TALIB RA & BASHA RK. 2021. Sourdough microbiome comparison and benefits. Microorganisms 9: 1355. https://doi.org/10.3390/microorganisms9071355.

LAZO-VÉLEZ MA, CAROCA-CÁCERES R, SUÁREZ-ESTRELLA D & SALDIVAR SO. 2021. Chapter 3 - Organic farming of wheat and sourdough quality. In Trends in Wheat and Bread Making. Charis M. Galanakis Eds, p. 67-94. https://doi.org/10.1016/B978-0-12-821048-2.00003-9.

LIU X, ZHOU M, JIAXIN C, LUO Y, YE F, JIAO S, HU X, ZHANG J & LU X. 2018. Bacterial diversity in traditional sourdough from different regions in China. LWT Food Sci Technol 96: 251-259. https://doi.org/10.1016/j.lwt.2018.05.023.

LYNCH KM, ZANNINI E, WILKINSON S, DAENEN L & ARENDT EK. 2019. Physiology of acetic acid bacteria and their role in vinegar and fermented beverages. Compr Rev Food Sci Food Saf 18: 587-625. https://doi.org/10.1111/1541-4337.12440.

MAHÉ F, ROGNES T, QUINCE C, DE VARGAS C & DUNTHORN M. 2015. Swarmv2: Highly-scalable and high-resolution amplicon clustering. PeerJ 3: e1420. https://doi.org/10.7717/peerj.1420.

MATSUSHIKA A, NEGI K, SUZUKI T, GOSHIMA T & HOSHINO T. 2016. Identification and characterization of a novel *Issatchenkia orientalis* GPI-anchored protein, IoGas1, required for resistance to low pH and salt stress. PLoS ONE 11: e0161888. https://doi.org/10.1371/journal.pone.0161888.

MCMURDIE PJ & HOLMES S. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8: e61217. https://doi.org/10.1371/journal.pone.0061217.

MENEZES LAA, MOLOGNONI L, POLÊNCIO LAS, COSTA FBM, DAGUER H & LINDNER JD. 2019. Use of sourdough fermentation to reducing FODMAPs in breads. Eur Food Res Technol 245: 1183-1195. https://doi.org/10.1007/s00217-019-03239-7.

MINERVINI F, LATTANZI A, DE ANGELIS M, CELANO G & GOBBETTI M. 2015. House microbiotas as sources of lactic acid bacteria and yeasts in traditional Italian sourdoughs.

Food Microbiol 52: 66-76. https://doi.org/10.1016/j.fm.2015.06.009.

NYANGA LK, NOUT MJR, GADAGA TH, THEELEN B, BOEKJOUT T & ZWIETERING MH. 2007. Yeasts and lactic acid bacteria microbiota from *masau* (*Ziziphus mauritiana*) fruits and their fermented fruit pulp in Zimbabwe. Int J Food Microbiol 120: 159-166. https://doi.org/10.1016/j.ijfoodmicro.2007.06.021.

OSHIRO M, TANAKA M, ZENDO T & NAKAYAMA J. 2020. Impact of pH on succession of sourdough lactic acid bacteria communities and their fermentation properties. Biosci Microbiota Food Health 39: 152-159. https://doi.org/10.12938/bmfh.2019-038.

PONTONIO E, ARORA K, DINGEO C, CARAFA I, CELANO G, SCARPINO V, GENOT B, GOBBETTI M & DI CAGNO R. 2021. Commercial organic versus conventional whole rye and wheat flours for making sourdough bread: safety, nutritional, and sensory implications. Frontiers Microbiol 12: 674413. https://doi.org/10.3389/fmicb.2021.674413.

PONTONIO E, RIZZELLO CG, DI CAGNO R, DOUSSET X, CLÉMENT H, FILANNINO P, ONNO B & GOBBETTI M. 2016. How organic farming of wheat may affect the sourdough and the nutritional and technological features of leavened baked goods. Int J Food Microbiol 239: 44-53. https://doi.org/10.1016/j.ijfoodmicro.2016.07.013.

POPA ME, MITELUT AC, POPA EE, STAN A & POPA VI. 2019. Organic foods contribution to nutritional quality and value. Trends Food Sci 84: 15-18. https://doi.org/10.1016/j.tifs.2018.01.003.

QUAST C, PRUESSE E, YILMAZ P, GERKEN J, SCHWEER T, YARZA P, PEPLIES J & GLÖCKNER FO. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res 41: D590-D596. https://doi.org/10.1093/nar/gks1219.

QUATTRINI M, LIANG N, FORTINA MG, XIANG S, CURTIS JM & GÄNZLE M. 2019. Exploiting synergies of sourdough and antifungal organic acids to delay fungal spoilage of bread. Int J Food Microbiol 302: 8-14. https://doi.org/10.1016/j.ijfoodmicro.2018.09.007.

REESE AT, MADDEN AA, JOOSSENS M, LACAZE G & DUNN RR. 2020. Influences of ingredients and bakers on the bacteria and fungi in sourdough starters and bread. mSphere 5: e00950-19. https://doi.org/10.1128/msphere.00950-19.

RIPARI V, GÄNZLE MG & BERARDI E. 2016. Evolution of sourdough microbiota in spontaneous sourdoughs started with different plant materials. Int J Food Microbiol 232: 35-42. https://doi.org/10.1016/j.ijfoodmicro.2016.05.025.

RIZZELLO CG ET AL. 2015. Organic cultivation of *Triticum turgidum* subsp. *durum* is reflected in the flour-sourdough fermentation-bread axis. App Environ Microbiol 81: 3192-3204. https://doi.org/10.1128/AEM.04161-14.

ROGNES T, FLOURI T, NICHOLS B, QUINCE C & MAHÉ F. 2016. VSEARCH: A versatile open source tool for metagenomics. PeerJ 4: e2584. https://doi.org/10.7717/peerj.2584.

SIEPMANN FB, ALMEIDA BS, RIPARI V, SILVA BJG, PERALTA-ZAMORA PG, WASZCZYNSKYJ N & SPIER MR. 2019. Brazilian sourdough: microbiological, structural, and technological evolution. Eur Food Res Technol 245: 1583-1594. https://doi.org/10.1007/s00217-019-03254-8.

SIMONETTI GD, OLIVO CJ, SEIBT DC, BRATZ VF, SAUTHIER JC & ADAMS CB. 2019. Productivity and nutritive value of elephant grass pastures under organic and conventional production systems. An Acad Bras Cienc 91: e20180011. http://dx.doi.org/10.1590/0001-3765201920180011.

UNITE. 2021. rDNA ITS based identification of Eukaryotes and their communication via DOIs. https://unite.ut.ee/ (accessed May 27, 2020).

URIEN C, LEGRAND J, MONTALENT P, CASAREGOLA S & SICARD D. 2019. Fungal species diversity in French bread sourdoughs made of organic wheat flour. Front Microbiol 10: 201. https://doi.org/10.3389/fmicb.2019.00201.

VAHER M, MATSO K, LEVANDI T, HELMIA K & KALJURAND M. 2010. Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties. Procedia Chem 2: 76-82. https://doi.org/10.1016/j.proche.2009.12.013.

VAN KERREBROECK S, MAES D & DE VUYST L. 2017. Sourdough as a function of their species diversity and processes conditions, a meta-analysis. Trends Food Sci Technol 68: 152-159. https://doi.org/10.1016/j.tifs.2017.08.016.

VOIDAROU C, ANTONIADOU M, ROZOS G, TZORA A, SKOUFOS I, VARZAKAS T, LAGIOU A & BEZIRTZOGLOU E. 2021. Fermentative foods: Microbiology, biochemistry, potential human health benefits and public health issues. Foods 10: 69. https://doi.org/10.3390/foods10010069.

VRČEK IV, CEPO DV, RASIC D, PERAICA M, ZUNTAR I, BOJIC M, MENDAS G & MEDIC-SARIC M. 2014. A comparison of the nutritional value and food safety of organically and conventionally produced wheat flours. Food Chem 143: 522-529. https://doi.org/10.1016/j.foodchem.2013.08.022.

WHITE TJ, BRUNS TD, LEE SB & TAYLOR JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: INNIS MA, GELFAND DH, SNINSKY JJ & WHITE TJ (Eds), PCR protocols: a guide to methods

and applications. Academic Press, San Diego, p. 315-322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1.

WICKHAM H, NAVARRO D & PEDERSEN TL. 2010. ggplot2: elegant graphics for data analysis. https://ggplot2-book.org/index.html. (accessed April 15, 2020).

YANG L, TAN Z, WANG D, XUE L, GUAN M, HUANG T & LI R. 2014. Species identification through mitochondrial rRNA genetic analysis. Sci Rep 4: 4089. https://doi.org/10.1038/srep04089.

YAZAR G & TAVMAN E. 2012. Functional and technological aspects of sourdough fermentation with *Lactobacillus sanfranciscensis*. Food Eng Rev 4:171-190. https://doi.org/10.1007/s12393-012-9052-1.

SUPPLEMENTARY MATERIALS

Figures S1-S3
Table SI

How to cite
COSTA LFX ET AL. 2022. Evolution of the spontaneous sourdoughs microbiota prepared with organic or conventional whole wheat flours from South Brazil. An Acad Bras Cienc 94: e20220091. DOI 10.1590/0001-3765202220220091.

Manuscript received on January 30, 2022;
accepted for publication on May 14, 2022

LETÍCIA F.X. COSTA[1]
https://oricd.org/0000-0001-8636-6449

CAROLINE ISABEL KOTHE[2]
https://oricd.org/0000-0003-3703-5699

TIELA T. GRASSOTTI[1]
https://oricd.org/0000-0003-1166-514X

RAQUEL P. GARSKE[3]
https://oricd.org/0000-0003-2705-751X

BEATRIZ N. SANDOVAL[3]
https://oricd.org/**0000-0002-5528-8617**

ANA PAULA M. VARELA[4]
https://oricd.org/0000-0003-3390-8691

JANIRA PRICHULA[4]
https://oricd.org/0000-0002-6767-8890

JEVERSON FRAZZON[3]
https://oricd.org/0000-0002-0209-1466

MICHELE B. MANN[1]
https://oricd.org/0000-0003-3335-8837

ROBERTA C.S. THYS[3]
https://oricd.org/0000-0002-1620-5006

ANA PAULA G. FRAZZON[1]
https://oricd.org/0000-0002-1029-5776

[1]Programa de Pós-Graduação em Microbiologia Agrícola e do Ambiente, Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Departamento de Microbiologia, Imunologia e Parasitologia, Rua Sarmento Leite, 500, 90050-170 Porto Alegre, RS, Brazil

[2]Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas, France

[3]Universidade Federal do Rio Grande do Sul, Instituto de Ciência e Tecnologia de Alimentos, Av. Bento Gonçalves, 9500, 91501-970 Porto Alegre, RS, Brazil

[4]Universidade Federal de Ciências da Saúde de Porto Alegre, Departamento de Ciências da Saúde Básicas, Rua Sarmento Leite, 245, 90050-170 Porto Alegre, RS, Brazil

Correspondence to: **Ana Paula Guedes Frazzon**
E-mail: ana.frazzon@ufrgs.br

Author contributions

Letícia da Fontoura Xavier Costa performed the methodologies and produced the manuscript; Caroline Isabel Kothe performed the bioinformatic analysis; Tiela Trapp Grassotti, Raquel Pischke Garske and Beatriz Nagel Sandoval contributed to experimental and statistical evaluations; Ana Paula Muterle Varela, Janira Prichula, Jeverson Frazzon, Michele Bertoni Mann and Roberta Cruz Silveira Thys contributed to improving the manuscript quality; Ana Paula Guedes Frazzon guided and assisted the project, manuscript and submission.