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Enhancing bread quality and extending shelf life using dried sourdough

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ABSTRACT

This study investigated the physicochemical properties, microbiological analysis, antifungal activity, and mycotoxin analysis of bread. Eight breads were prepared, including control Bread (CB), control Bread with calcium propionate (CPB), commercial sourdough breads (CCB), control bread made with oven-dried sourdough (SB), and bread baked with oven-dried sourdough fermented by *Pediococcus pentosaceus* TI6 (SB-TI6). These breads were contaminated with *Aspergillus flavus* and *Penicillium verrucosum*, and their shelf life was monitored over 7 days. The results indicated that incorporating sourdough powder into bread significantly extends the shelf life and reduces fungal contamination. This reduction resulted in lower levels of mycotoxins, with the use of dry sourdough fermented by *P. pentosaceus* TI6 leading to the lowest levels of aflatoxins. In the case of SB-TI6, minor levels of aflatoxin B1 (AFB1), B2 (AFB2), and G1(AFG1) were detected, with only 0.003 mg of AFG2/Kg of the product. Conversely, CB loaves exhibited the highest mycotoxin contamination levels, reaching values of 330 mg of AFB1/Kg. The findings suggested that incorporating dry sourdough in breadmaking served as a viable alternative to synthetic preservatives, effectively reducing mycotoxin levels and enhancing bread safety and shelf life.

1. Introduction

Fungi and yeast, commonly found in the environment, are often integral to the natural flora of foods, playing beneficial roles in food production. However, under certain conditions, they may also contribute to product deterioration (Niego et al., 2023). Mold can grow in several types of foods, such as cereals, meat, milk, and fruits, causing bad smells, discoloration, and toxin production (El Sheikha & Mahmoud, 2015). Particularly, fungal genera, such as *Penicillium*, *Aspergillus*, and *Fusarium* are primary contaminants found in cereal-based products and are known to produce mycotoxins, such as aflatoxins (AF) and ochratoxins (OTA), which pose significant health risks, as documented by Noroozi et al. (2022) and Omotayo, Omotayo, Mwanza, and Babalola (2019). AF, classified as Group 1 by the International Agency for Research on Cancer (IARC), is linked to mutagenic and carcinogenic effects due to the formation of DNA adducts. Additionally, OTA is known to impact the immune system and is classified as a possible human carcinogen (Bui-Klimke & Wu, 2015).

Certain bakery products, particularly bread, are vulnerable to spoilage due to their composition. The combination of high humidity and near-neutral pH levels creates an environment favorable to contamination during storage (Axel, Zannini & Arendt, 2017). Although baking at high temperatures can eliminate some spores, post-baking contamination remains a common occurrence. Therefore, it is crucial to employ effective techniques to prevent fungal growth (Pétel, Onno, & Prost, 2017). Currently, chemical preservatives are employed in bread making to inhibit fungal growth. However, consumers' apprehension regarding the excessive use of chemical additives, such as ethanol, calcium propionate, and sorbate has promoted a demand for natural alternatives (Iosca et al., 2023). The use of sourdough as a biopreservative technique has gained traction due to its ability to improve the technological properties of milled cereals, especially in bread production. Sourdough, a lacto-fermented flour with acidic properties, contains lactic acid bacteria (LAB) that play a crucial role in developing sour characteristics during the fermentation process (Bartkiene, Özogul, & Rocha, 2022)

Likewise, the fermentation of sourdough by LAB acidifies the medium, inhibiting the growth of pathogenic microorganisms. Thus, sourdough contributes to the enhancement of bread quality, including its shelf life, flavor, and texture (Arsoy, Gül, & Çon, 2022). LAB presence in sourdough not only facilitates dough fermentation but also modifies various compounds and nutrients. This microbial activity results in the production of health-beneficial substances and compounds with antifungal properties, including organic acids, volatile organic acids,

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hydrogen peroxide, fatty acids, and bacteriocins (Pavlidis, Mallouchos, Ercolini, Panagou, & Nychas, 2019).

The process of sourdough preparation involves multiple refreshment steps, which contribute to the development of a stable microbiome of microorganisms, which is the so-called batch-sloping procedure. This well-established microbiome can then be used as a leavening agent in sourdough bread production. Despite the convenience of using commercial bakers' yeasts, sourdough offers several advantages. It enhances bread rheology, extends shelf life, and provides superior nutritional properties compared to bread made merely with commercial yeasts (Warburton, Silcock, & Eyres, 2022).

Previous research has delved into harnessing the antifungal properties of LAB isolated from diverse sourdoughs, aiming to prepare a dry sourdough suitable for incorporation into breadmaking processes (Lafuente et al., 2023). Among the array of starter cultures examined, the *Pediococcus pentosaceus* TI6 strain emerged as a stand-out performer, exhibiting superior antifungal efficacy *in vitro* and demonstrating heightened production of antifungal compounds during sourdough fermentation. Furthermore, to facilitate sourdough utilization in breadmaking, dried sourdough fermented using the *P. pentosaceus* strain subjected to drying at 40 °C emerged as the optimal conditions for future applications. This choice yielded a powder boasting enhanced antifungal activity, microorganism viability, and optimal product yield. The findings underscore the importance of considering such optimized parameters for subsequent industrial-scale applications.

Against this backdrop, this study aimed to investigate the antifungal and antitoxigenic properties of breads produced using dried sourdough fermented by *P. pentosaceus* TI6, comparing these properties with those of conventional breads. Additionally, the study sought to characterize the antifungal compounds present in the dried sourdough throughout the fermentation and post-baking stages, providing a comprehensive analysis of the potential benefits of using dried sourdough in bread production.

2. Material and methods

2.1. Chemicals and ingredients

The culture media used included Man Rogosa Sharpe Broth (MRS), Man Rogosa Sharpe Agar (MRS), Potato Dextrose Broth (PDB), Potato Dextrose Agar (PDA), and Rose Bengal Chloramphenicol Agar, all obtained from Liofilchem Bacteriology Products (Roseto Degli Abruzzi, Italy). Deionized water (<18 MΩ/cm of resistivity) used to make the culture media was obtained by a Mili-Q purification system (Millipore, Bedford, USA).

Reagents, such as tween 80 and glycerol, were from Sigma-Aldrich (St. Louis, USA). Solvents used for liquid chromatography were sulfuric acid, ethyl acetate, acetonitrile (ACN), and methanol (MeOH) from VRM Chemicals (Radnor, PA, USA). Sodium carbonate and sodium hydroxide were acquired from Fisher Scientific (Loughborough, UK).

Concerning the elaboration of the sourdoughs and subsequent preparation of bread for the study, wheat flour, salt, and sugar from the Hacendado brand (Mercadona, Burjassot, Valencia, Spain), fresh yeast from Levanova brand (Masymas, Burjassot, Valencia, Spain) and Bezoya mineral water (Mercadona) were used. In addition, to make a control bread, Calcium propionate from Sigma-Aldrich (St. Louis, MO, USA) was added. To provide a basis for comparison in the study, two sourdough commercial products were purchased from the online shopping site Amazon and included in the dough elaboration, labeled as commercial product 1 *Masa mater* (CCB 1) and commercial product 2 *Levanova* (CCB 2). These commercial products were commercial sourdough starters sold in powdered form, which were added to bread-making processes as leaving agents. The nutritional composition of the wheat flour was 73% carbohydrates, of which 0.6% was sugar, 10% protein, 1.3% fat (including 0.1% saturated fat), 3% fiber, and 0.02% salt.

2.2. Selection of the dry sourdough

The *Pediococcus pentosaceus* TI6 was activated in MRS broth at 37 °C for 24 h and recultured in 100 mL of MRS broth for 24 h. After, the suspension was centrifuged at 3300×*g* at 4 °C for 10 min. Next, the supernatant was rejected, and the pellet was resuspended in 125 mL of sterile mineral water and mixed with 100 g of sterile wheat flour to generate a final inoculum of 10^7 CFU/g. The flour was sterilized by placing it inside a glass jar to avoid contact with water. Then, the doughs were fermented and fed using the back-slopping method every 48 h. The doughs were kept at 25–28 °C until they were refreshed with 50 mL of sterile mineral water and 50 g of sterile wheat flour. After seven days of fermentation, the dough was extended and dried to obtain a valuable dry sourdough for bread making. These drying techniques included atomization, lyophilizing, reducing humidity during drying, and drying in an oven at 40 °C. As previously described by Lafuente et al. (2023), doughs were fermented at 25–28 °C, refreshing them with 50 mL sterile water and 50 g of wheat flour, and drying them to create a valuable dry sourdough for bread making. The best performance was achieved by drying the dough at 40 °C for 24 h in an oven in terms of antifungal activity, viability of the microorganism, and yield of the product weight.

2.3. Bread elaboration

For the study, the breads were processed and sliced. The groups of samples were divided as follows: control loaves without the addition of sourdough (CB) and a control group with calcium propionate (CPB). The CCB 1 group and CCB 2 group corresponded to commercial products used to make sourdough bread. The bread corresponding to SB 10% and SB 20% were elaborated with 10 g/100g and 20 g/100g of dried sourdough, respectively, without inoculated bacteria. And finally, bread 7 (SB-TI6 10%) and bread 8 (SB-TI6 20%) are the breads made with sourdough dried at 40 °C fermented by LAB *P. pentosaceus* TI6 at 10% and 20%, respectively (Table 1).

All the breads were made with wheat flour, mineral water, sugar, fresh yeast, and the amount of salt permitted by legislation (1.31 g/100g), except for commercial products, which followed their specific protocol. Two bowls were prepared for the CCB1 product: the first contained 500 g of flour, 300 g of water, and one small sachet of 5 g; the second contained a larger sachet of 100 g, plus 100 g of water and 10 g of salt. Bowl 1 was kneaded and allowed to rest for 30 min before being mixed with the contents of Bowl 2. The CCB2 product was prepared by adding a sachet of 10 g per 500 g of dough, then the mixture was the remaining ingredients and knead.

Warm water was mixed in a glass with the fresh yeast and sugar. Subsequently, this mixture was introduced into the bread maker (Princess 152006; Tilburg, Nederland) along with the wheat flour and the salt and left to knead for approximately 5 min. Once finished, part of this dough (5 g of each dough per triplicate for each analysis) was sampled, corresponding to the prefermented dough (t_0), and the rest of the dough (200 g) was introduced into molds and left to ferment, covering with a damp cloth. Each type of bread underwent fermentation for different durations: CB and CPB for 1 h, CCB 1 for 3 h, CCB 2 for 2 h, SB 10%, SB 20%, SB-TI6 10%, and SB-TI6 20% for 12 h. These varying proofing times were primarily dictated by the lower yeast content and the nature of the microorganisms involved, particularly LAB. LAB typically require longer to effectively influence the dough properties due to their metabolic characteristics. After this fermentation period, the required quantities were extracted for analysis (t1) and subsequently baked at 200 °C for 45 min. Before sampling for analysis, the baked bread was allowed to cool to room temperature under controlled, sterile conditions. Analyses were conducted in triplicate for each treatment at various time points. Fig. 1 summarizes the elaboration and sampling process.

In compliance with Spanish legislation (Real Decreto 308/2019) regarding the quality standard for bread, the sourdough used in this

Table 1
Percentage composition of bread ingredients.

	Ingredients (%)							
	Wheat flour	Mineral water	Yeast	Salt	Sugar	Calcium propionate	Dry sourdough	Commercial product
CB	60	32.7	4	1.3	2	–	–	–
CPB	59.7	32,7	4	1.3	2	0.30	–	–
CCB 1	47.7	47.7	–	1.3	–	–	–	3.3
CCB 2	49.3	39.4	–	1	–	–	–	10.3
SB 10%	54	32.7	0.1	1.2	2	–	10	–
SB 20%	44	32.7	0.1	1.2	2	–	20	–
SB-TI6 10%	54	32.7	0.1	1.2	2	–	10	–
SB-TI6 20%	44	32.7	0.1	1.2	2	–	20	–

CB: control bread; CPB: bread control with propionate; CCB 1: commercial sourdough bread 1; CCB 2: commercial sourdough bread 2; SB 10%: sourdough bread with control sourdough inoculum (10 %); SB 20%: sourdough bread with control sourdough inoculum (20 %); SB-TI6 10%; sourdough bread with *P. pentosaceus* TI6 inoculum (10%); SB-TI6 20%; sourdough bread with *P. pentosaceus* TI6 inoculum (20%).

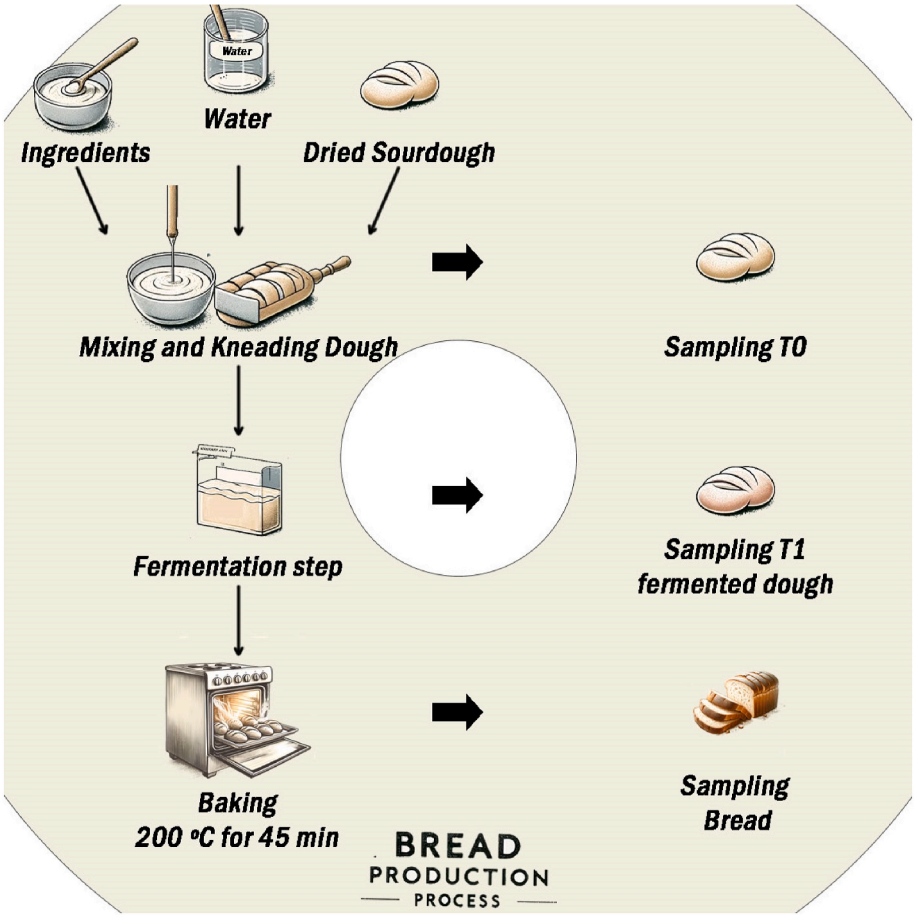

Fig. 1. Bread production process and sampling time points.

study was confirmed to contain an acidifying microflora of living LAB, specifically the *P. pentosaceus* strain. After dehydration and rehydration, the sourdough maintained a viable bacterial flora, ensuring effective fermentation of the dough as demonstrated in Table 2. According to the legislation, bread labeled as made with sourdough must contain no more than 0.2% industrial yeast relative to the total weight of the flour. Our data confirmed the presence of active microorganisms at various stages of dough preparation and bread making, indicating that both the commercial sourdough products and the sourdough fermented by *P. pentosaceus* are active, with living microorganisms contributing to the fermentation process and extended shelf life.

2.4. Determination of volatile organic compounds (VOCs) of the Dehydrated sourdoughs fermented by P. pentosaceus TI6

The VOCs in the dried sourdoughs were determined through a microextraction in the solid phase of the headspace (HS-SPME) and analyzed with gas chromatography with a single-quadrupole mass spectrometer detector (GC/MS) following the method described by Warburton et al. (2022) with modifications.

Five grams of each sample were added to 20 mL of MiliQ water in a vial to analyze the samples. The vials underwent a 5-min preincubation period in a water bath at 50 °C, and the fiber was exposed for 45 min under continuous agitation. The fiber used to absorb the VOCs was a DVB/C-WR/PDMS coated SPME fiber (80 μm × 10 mm) (Supelco,

Table 2
Results of physical-chemical parameters in the samples before and after fermentation, and in the final product.

Properties	Dough T0							
	CB	CPB	CCB 1	CCB 2	SB 10%	SB 20%	SB-TI6 10%	SB-TI6 20%
a) pH	5.44 ± 0.15^{c}	5.98 ± 0.05^{e}	5.68 ± 0.06^{d}	4.32 ± 0.03^{a}	6.07 ± 0.03^{e}	5.00 ± 0.04^{b}	5.65 ± 0.03^{d}	4.91 ± 0.07^{b}
b) Titratable acidity (mL NaOH)	6.00 ± 0.00^{b}	10.03 ± 0.81^{a}	5.67 ± 0.15^{b}	2.97 ± 0.06^{cd}	2.50 ± 0.10^{d}	3.53 ± 0.06^{c}	2.13 ± 0.06^{d}	4.67 ± 0.06^{c}
c) Total microorganisms (Log (CFU/g))	7.66 ± 0.01^{bc}	7.77 ± 0.01^{a}	7.64 ± 0.02^{bc}	7.30 ± 0.26^{c}	7.63 ± 0.04^{bc}	7.76 ± 0.02^{bc}	7.88 ± 0.01^{b}	7.93 ± 0.03^{b}
d) Yeasts (Log (CFU/g))	7.40 ± 0.11^{b}	8.76 ± 0.03^{a}	ND	ND	ND	ND	ND	ND
	Fermented dough							
	CB	**CPB**	**CCB 1**	**CCB 2**	**SB 10%**	**SB 20%**	**SB-TI6 10%**	**SB-TI6 20%**
a) pH	5.52 ± 0.09^{ab}	5.59 ± 0.08^{a}	5.39 ± 0.08^{b}	4.16 ± 0.02^{e}	5.43 ± 0.05^{ab}	4.75 ± 0.06^{c}	4.78 ± 0.02^{c}	4.51 ± 0.09^{d}
b) Titratable acidity (mL NaOH)	6.13 ± 0.21^{bc}	7.93 ± 0.29^{a}	5.97 ± 0.21^{c}	6.00 ± 0.10^{bc}	3.83 ± 0.15^{e}	4.40 ± 0.17^{d}	4.10 ± 0.10^{d}	6.50 ± 0.26^{b}
c) Total microorganisms (Log (CFU/g))	7.35 ± 0.17^{d}	8.63 ± 0.01^{bc}	8.97 ± 0.01^{bc}	7.26 ± 0.20^{d}	7.44 ± 0.09^{d}	7.96 ± 0.01^{c}	9.08 ± 0.05^{b}	9.27 ± 0.02^{a}
d) Yeasts (Log (CFU/g))	7.51 ± 0.05^{b}	8.49 ± 0.02^{a}	8.61 ± 0.06^{a}	7.47 ± 0.01^{b}	7.59 ± 0.02^{b}	7.48 ± 0.01^{b}	8.55 ± 0.01^{a}	8.54 ± 0.09^{a}
	Bread							
	CB	**CPB**	**CCB 1**	**CCB 2**	**SB 10%**	**SB 20%**	**SB-TI6 10%**	**SB-TI6 20%**
a) pH	5.39 ± 0.04^{a}	5.31 ± 0.10^{a}	5.30 ± 0.10^{a}	5.33 ± 0.10^{a}	4.88 ± 0.06^{b}	4.92 ± 0.08^{b}	4.80 ± 0.11^{bc}	4.69 ± 0.02^{c}
b) Titratable acidity (mL NaOH)	6.60 ± 0.10^{a}	4.27 ± 0.06^{c}	4.67 ± 0.15^{c}	6.33 ± 0.06^{a}	3.33 ± 0.06^{d}	5.20 ± 0.10^{b}	5.97 ± 0.06^{b}	6.50 ± 0.06^{a}
c) Specific volume (mL/g)	225 ± 0.00^{b}	235 ± 0.00^{b}	235 ± 0.00^{b}	330 ± 0.00^{b}	250 ± 0.00^{ab}	250 ± 0.00^{ab}	275 ± 0.00^{a}	280 ± 0.00^{a}
d) a_w	0.79 ± 0.01^{ab}	0.84 ± 0.00^{a}	0.81 ± 0.04^{a}	0.71 ± 0.06^{bc}	0.60 ± 0.03^{e}	0.65 ± 0.04^{de}	0.67 ± 0.03^{cd}	0.63 ± 0.04^{e}
e) Moisture content (g/100g)	30.40 ± 0.09^{a}	30.49 ± 0.10^{a}	30.14 ± 0.46^{a}	30.03 ± 0.34^{a}	30.54 ± 0.07^{a}	30.52 ± 0.03^{a}	30.03 ± 0.36^{a}	30.26 ± 0.10^{a}
f) Total microorganisms (Log (CFU/g))	ND	ND	5.51 ± 0.05^{b}	5.45 ± 0.03^{b}	ND	ND	5.76 ± 0.03^{ab}	5.89 ± 0.07^{a}
g) Yeasts (Log (CFU/g))	ND	ND	ND	ND	ND	ND	ND	ND

CB: control bread; CPB: bread control with propionate; CCB 1: commercial sourdough bread 1; CCB 2: commercial sourdough bread 2; SB 10%: sourdough bread with control sourdough inoculum (10%); SB 20%: sourdough bread with control sourdough inoculum (20%); SB-TI6 10%; sourdough bread with *P. pentosaceus* TI6 inoculum (10%); SB-TI6 20%; sourdough bread with *P. pentosaceus* TI6 inoculum (20%). Different lower-case letters indicate significant differences between samples ($p \geq 0.05$).

Bellefonte, PA, USA). After 45 min, the fiber was introduced into an Agilent 7890A gas chromatograph coupled to an Agilent 7000A triple quadrupole mass spectrometer equipped with an electronic impact source (EI). The desorption was performed at 250 °C for 10 min, and injection was performed in spitless mode. The chromatographic separation was performed using a column HP-5MS (30 m × 0.25 mm, 0.25 μm) from J&W Scientific (Folsom, CA, USA).

Moreover, the following temperature ramp was set. First, the program started at 40 °C for 2 min and increased to 160 °C at 6 °C/min after boosting the temperature to 260 °C at 10 °C/min and keeping it constant for 40 min. The flow of the carrier gas, 99.99% helium, was 2.5 mL/min. The detection of the compounds was carried out in a range m/z of 40–450 Da in full scan mode. The compounds were identified through the NIST Atomic Spectra Database version 1.6 (Gaithersburg, MD, USA) using 95% spectral similarity. The linear retention index (LRI) was calculated regarding the time retention of an alkane solution (C8–C20) analyzed with the same conditions as the samples. The proportion of VOC in the sample was calculated by dividing the analyte area by the total pick area of identified compounds.

2.5. Physicochemical analyzes and microbiological properties of doughs and bread

To know the effect of adding sourdough fermented by LAB, *P. pentosaceus* TI6, and its subsequent drying in the preparation of bread, its properties have been studied. Samples of the doughs of the 8 breads prepared have been taken at three different time points: at t_0, after making the dough, at t_1, after the dough fermentation; and after baking the bread (t2).

First, the changes in pH and titratable acidity (TA) were studied. For this, 10 g of sample were taken in triplicate at each moment, and later, with the help of a solid's pH meter, the pH was measured. Taking advantage of the pH test sample, the TA was measured. To the 10 g sample, 45 mL of deionized water was added, and it was homogenized using the Ultraturrax (Ultra Ika T18 basic Staufen, Germany). After homogenization, 0.1 M NaOH was added until the pH changed to 8.5 and the mL of 0.1 M NaOH added was recorded (Siepmann, De Almeida, Gomes, Waszczynskyj, & Spier, 2022).

After baking the bread, their specific volume was calculated using the millet seed displacement method (Mannuramath, Yenagi, & Orsat, 2015). The breads were placed in a beaker filled with chia seeds, and the difference in volume of the same amount of chia seeds in the beaker with and without the bread was used for volume calculation. The breads were then weighed, and the specific volume was calculated as the ratio between volume and weight of the samples. The study of the water activity (a_w) of the bread was also realized using the Humimeter RH2 water activity meter (Max-Schaller-Straße, Austria). First, slices of bread were cut to be able to place them in the meter, and the a_w was determined employing the Humimeter RH2 for 3 min.

To evaluate the humidity of the product, the product was dried in a hot air oven (Calvo-Carrillo, López-Méndez, Carranco-Jáuregui, & Marines, 2020). It consists of measuring the weight of the product after the evaporation of water from the loaves. The subtraction of the weights determines the moisture of the product. For this, the weight of different crucibles was taken and about 10 g of bread was placed in them. Next, they were placed in an oven at 100 °C for 1 h. After drying, the samples were weighed and with the difference in weight, the humidity of the product was obtained, which is expressed in percentage of the evaporated weight.

Regarding the microbiological analysis, a count of yeasts and bacteria was performed in t0, t1, and bread as described in Luz et al. (2021) with modifications adapted to this study. For cell count 10 g of each dough was diluted with peptone water (1 g/L) and the seeding on MRS Agar to count the viability of LAB and Rose Bengal Chloramphenicol

Agar for yeasts and then maintained at 37 °C and 25 °C respectively for 48 h.

2.6. Determination of organic acids in the doughs and breads

The methodology defined by Lafuente et al. (2023) was used to determine the lactic acid content of the breads and doughs. Deionized water was used to dilute the samples 1:20, and an Ultraturrax (Ultra Ika T18 basic Staufen, Germany) was used to homogenize them. Following homogenization, a 0.22 μm pore size cellulose filter membrane was used to filter the materials. The filtered samples were then added to the Agilent 1100 Series HPLC System, Santa Clara, CA, USA, a high-performance liquid chromatography system. The HPLC was furnished with a Rezex ROA-Organic Acid (150 mm × 7.8 mm) reverse phase column (Phenomenex Inc., Torrance, CA, USA), a diode array detector Jasco MD-4015 (Easton, MD, USA), a Jasco PU-4180 pump (Easton, Maryland, USA), and 20 μL of the sample injection. A 0.005 M sulfuric acid solution in water was used as the mobile phase, and it was pumped at a rate of 0.8 mL/min for 10 min at 40 °C. The detectors' wavelength of 214 nm was chosen to measure organic acids. Samples were evaluated in triplicate, and the calibration curve was run using lactic acid dissolved in water at values ranging from 0 to 1000 mg/L. The software utilized for data collection was ChromNav 2.0 HPLC (Jasco, Easton, MD, USA), and the results were expressed in g/kg.

2.7. Antifungal activity of the bread

To analyze the antifungal activity of the prepared breads, the method described by Debonne, Van Bockstaele, De Leyn, Devlieghere, and Eeckhout (2018) with modifications adapted to our study was performed. From the baked bread, 4 slices of bread were taken in duplicate and 4 spots of 10 μL were inoculated with a suspension of spores of the fungi *A. flavus* and *P. verrucosum*. Once the slices were contaminated with a concentration of 10^4 spores/g of each fungus they were stored in sterile bags. Once the days of visualization of the fungal growth had elapsed, microbiological analysis of the contaminated slices of bread was made. Two of the four sections were taken, and a 1:10 (m/v) dilution was made with peptone water (1 g/L) and tween 80 (1 mL/L) and mixed with the Stomacher. Subsequently, serial dilutions were made and 100 μL were seeded with a Drigalsky loop in PDA plates and incubated at 25 °C for 48 h.

2.8. Mycotoxins analysis of contaminated breads by fungi

The methodology used by Luz et al. (2018) with modifications was performed in order to determine the mycotoxin content of the bread contaminated by *A. flavus* and *P. verrucosum*. After 7 days, the contaminated breads were weighed in 5 g in a Falcon tube with 25 mL of MeOH, then the samples were homogenized for 5 min using an Ultraturrax (Ultra Ika T18 basic Staufen, Germany). After being stirred for 24 h, the samples were centrifuged at 3300×*g*, for 10 min at 4 °C and the supernatant was evaporated with Büchi Rotavapor R-200 (Postfach, Switzerland). At last, 1 mL of MeOH was added to the dried samples and then filtered using 0.22 μm filters and in the last place samples were vialized before the analysis with UHPLC (1290 Infinity LC, Agilent Technologies) coupled to a Q-TOF (Agilent 6540 LC/QTOF) Mass Spectrometer.

The Agilent Zorbax RRHD SB-C18 (2.1 × 50 mm, 1.8 μm) column allowed the chromatographic separation of the mycotoxins. The mobile phase (Phase A) used was composed of Milli-Q water 1 mL/L FA and the mobile phase (Phase B) by ACN 1 mL/L FA. The configuration of the gradient employed was: 0 min, 2% B; 22 min, 95% B; 25 min, 5% B. Afterward, the column was equilibrated 3 min before the next injection. The flow rate used was 0.4 mL/min, and the injection volume was established at 5 μL. For Q-TOF analysis, an Agilent Dual Jet Stream electrospray ionization (ESI) worked in positive ionization mode. The established conditions of ESI were the following ones: gas temperature of 325 °C; gas flow of 10 L/min; nebulizer pressure of 40 psig; sheath gas flow of 12 L/min; sheath gas temperature of 295 °C; capillary voltage of 4000 V; skimmer of 70 V; nozzle voltage of 500 V; scan range: 100–1500 Da and collision energy: 10, 20, 40 eV. For quantification, an aflatoxin calibration curve was elaborated, with a range of concentration of 0.01–10 mg/L. MassHunter Qualitative Analysis Software B.08.00 allowed the collection of data analyzed.

2.9. Statistical analyses

The analyses were realized in triplicates and the mean values and standard deviation were estimated. The Software employed for the statical analysis was InfoStat 2019 (Universidad Nacional de Córdoba, Córdoba, Argentina). The significant differences in the data were determined using Tukey's multiple comparison test. The significance level was at $P \leq 0.05$.

3. Results and discussion

3.1. Determination of VOCs of the dried sourdoughs fermented by P. pentosaceus TI6

Four different techniques were employed to dry the sourdoughs, and the resulting VOCs profile was characterized using the headspace solid-phase microextraction method. The profile of VOCs in the samples can be attributed to the LAB's metabolic activity during sourdough fermentation and the drying technique employed. A total of forty VOCs were identified, including alcohols (7), aldehydes (14), alkanes (3), esters (4), ketones (2), terpenes (9), and thiazoles (1).

Different types of sourdoughs demonstrate distinct profiles of VOCs, with the complexity of the generated compounds being influenced by various reactions throughout the fermentation process, thermal fluctuations, enzymatic responses, and lipid oxidation (Pétel et al., 2017). During fermentation, VOCs are produced, primarily comprising acids, esters, ketones, and alcohols. Additionally, reactions, such as lipid oxidation primarily yield aldehydes and ketones, while the Maillard reaction contributes to the formation of compounds like pyridines, pyrroles, and pyrazines (Illueca et al., 2023; Pétel et al., 2017). Likewise, when sourdough is incorporated into bread-making, it promotes the development of volatile compounds that impart distinctive aromas and flavors to the final product (Wu et al., 2022). Therefore, the method employed for drying the sourdough can impact the diversity of the VOCs present since volatile compounds can be generated through the Maillard reaction, while other volatile compounds may be lost during the drying process.

As observed in Fig. 2, different profiles of VOCs were observed depending on the applied drying technique. The highest variety of VOCs was found in the sourdough fermented with *P. pentosaceus* TI6 and dried at 40 °C in an oven. In comparison to the other sourdoughs, this sourdough exhibited a significant number of compounds. The most abundant compounds generated were aldehydes, with a notable presence of nonanal, 2−nonenal, 4−nonenal, and heptanal. Additionally, the production of limonene, a terpene, was also prominent. Despite lower levels of alcohol production, 2−undecanol, 1−octanol, and 1−octen−3−ol were primarily detected.

On the other hand, in the spray-dried sourdough, the presence of terpenes and aldehydes, such as limonene, terpinene-4-ol, citronellol, β-phellandrene, and decanal, stood out. In the lyophilized sample, a higher content of aldehydes, such as decanal and hexanal, as well as alcohols like 1-hexanol, could be observed. Finally, the sourdough dried to low humidity exhibited a higher presence of alcohols, including 1-hexanol, 2-ethyl, and 2-undecanol.

Among the aldehydes that were generated in the oven-dried sourdough, the presence of nonanal, 2−nonenal, 4−nonenal, and heptanal was particularly notable. Nonanal can be produced during the

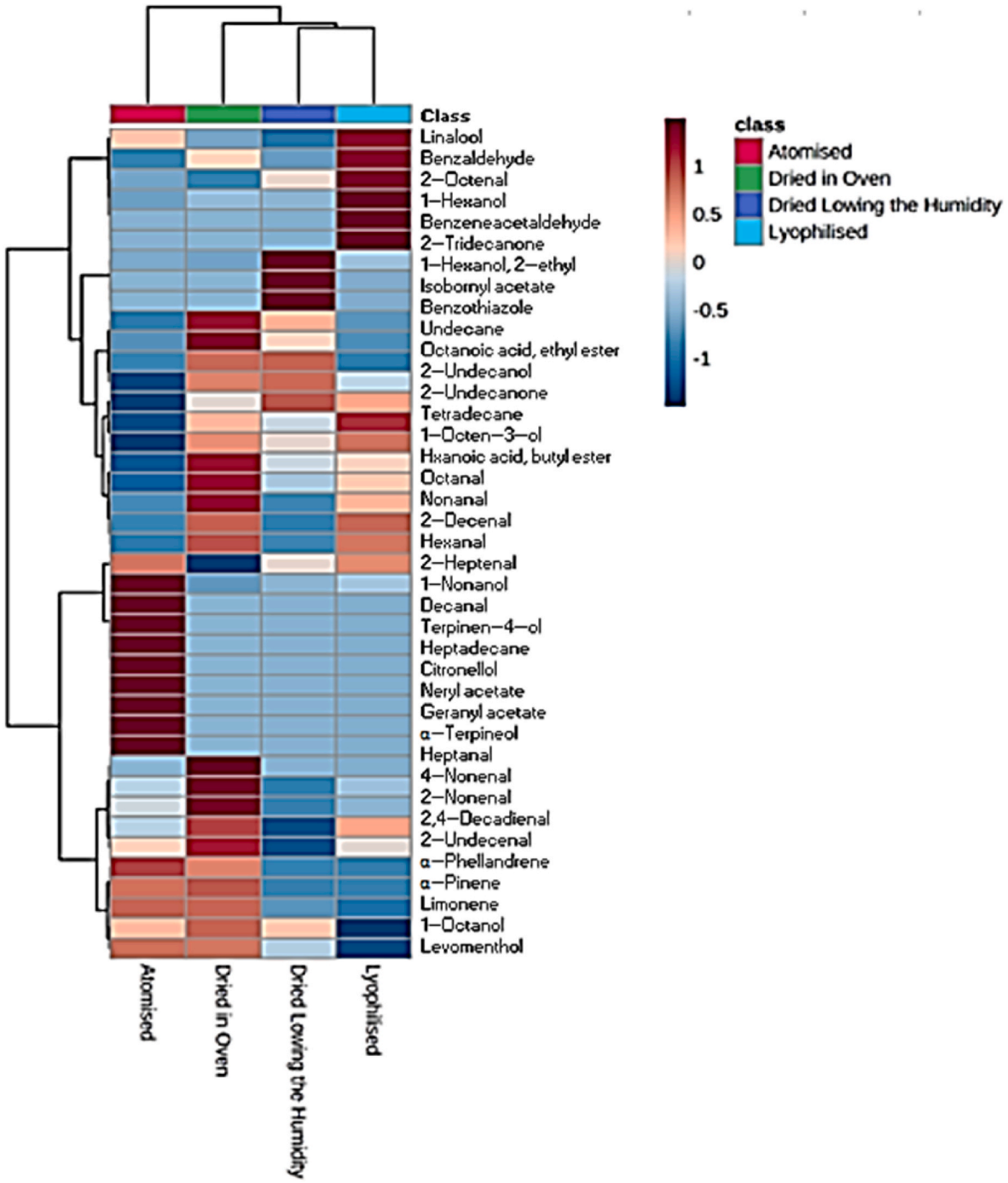

Fig. 2. Heatmap of the abundance of the volatile organic compounds produced in the sourdoughs fermented with *Pediococcus pentosaceus* TI6 and dried using different methods: atomized sample, lyophilized, dried by reducing humidity, and dried in the oven at 40 °C.

fermentation of sourdough by specific strains of lactic acid bacteria, including *P. pentosaceus* (Shen et al., 2022). LAB, responsible for the fermentation process in sourdough, possesses the ability to convert oleic acid into nonanal through a series of metabolic reactions (Păucean et al., 2021). On the other hand, 2−nonenal and 4−nonenal are generated through the oxidation of linoleic and linolenic acids, which are fatty acids found in flour (Ishino et al., 2010). The presence of nonanal, 2−nonenal, 4−nonenal, and heptanal contributes to the distinctive aroma and flavor of sourdough bread, and they are also known for their antimicrobial properties.

A study conducted by Li, Zhu, Xie, and Liang (2021) examined the impact of aldehydes, including nonanal, octanal, and decanal, on their antifungal effects against *A. flavus* and certain *Penicillium* spp. The research revealed that these aldehydes exerted antifungal activity by causing damage to the chitin, a key component in fungal cell walls. Another significant volatile compound present in the sourdough dried at 40 °C is heptanal. Recent findings, such as those by Li et al. (2021), have demonstrated that heptanal also possesses antifungal properties against *A. flavus*. Heptanal induces damage to the plasma membrane, disrupts mitochondrial activity, and triggers oxidative stress within the fungal cells.

Limonene, a cyclic terpene, was found to be another predominant volatile compound identified in the sourdough, and it is commonly found in citrus fruits (Magalhães, Vilas-Boas, Teixeira, & Pintado, 2023).

Although limonene is not primarily produced during sourdough fermentation, it can be formed during the baking process when the heat triggers the oxidation of terpenes present in the flour (Galoburda, Straumite, Sabovics, & Kruma, 2020). Since the bread-baking process was not performed in this case, it can be inferred that the presence of limonene may be attributed to the drying process of the dough. While there is limited research specifically focused on the antifungal activity of limonene in sourdough, some studies suggest that it has the potential to act as a natural antifungal agent (Ribeiro et al., 2023; Sosa et al., 2023).

Valková et al. (2021) conducted a study investigating the antifungal activity of *Citrus reticulata* essential oil, which primarily contains limonene, against *Penicillium* spp. The authors reported promising results in terms of inhibiting the growth of the fungi. Similarly, Ferna (2008) conducted a study exploring the antifungal activity of citrus essential oils, including limonene, against various fungal species, such as *A. flavus* and *P. verrucosum*. The essential oils demonstrated strong antifungal activity against certain fungi. In a separate study by dos Reis Gasparetto et al. (2022), the essential oil extracted from Tahiti lemon peel was investigated, and the findings highlighted the relationship between the antifungal capacity and the presence of volatile compounds, particularly limonene.

On the other hand, although the concentrations of alcohols in the volatile profile were relatively lower, the samples demonstrated significant prevalence of 2−undecanol, 1−octanol, and 1−octen−3−ol. The production of these volatile organic compounds (VOCs) in sourdough may be attributed to the activity of LAB and yeasts.

Despite the lower concentrations of alcohols in the volatile profile, the presence of 2−undecanol, 1−octanol, and 1−octen−3−ol was highlighted. These VOCs were likely produced because of the combined action of LAB and yeasts in the sourdough.

Suo et al. (2020) conducted a study to investigate the effect of using traditional Chinese starters, characterized by the predominance of *Lactiplantibacillus* spp. and *Pediococcus* spp., on the volatile profile of steamed bread. In their research, they identified the presence of 1−octen−3−ol in the bread. This compound, known for its characteristic flavor and aroma, contains a double bond that contributes to the unique sensory attributes of the product.

3.2. Dry sourdough effect on the characterization of dough and bread

The results presented in Table 2 showed the effects of incorporating dry sourdough into the bread-making process, as well as the properties of the dough at different stages, i.e., freshly made (t0), fermented, and the final bread product. The results indicated significant differences between the various doughs and breads.

Specifically, the doughs and breads prepared with dry sourdough fermented by *P. pentosaceus* TI6 (SB-TI6 20%) exhibit the lowest pH values, indicating a higher acidity in these samples compared to the control samples. Similarly, the bread made with the commercial product CCB 2 also demonstrates low pH levels, which can be attributed to its powder content. Throughout the fermentation process and in the final product, these breads maintain the lowest pH, primarily due to the activity of the dry sourdough inoculated with LAB *P. pentosaceus* TI6. The inoculation of LAB and subsequent production of various organic acids through its metabolic processes result in increased acidity, thereby contributing to an extended shelf life of the product. Consequently, the pH and TA of sourdough bread are critical factors influencing its overall quality and longevity (Katsi et al., 2021).

To ensure the quality and longevity of the sourdough breads, we measured both the TA and pH of the sourdough samples. The results indicated that CCB2 exhibited a higher concentration of lactic acid compared to CCB1, both at the initial stage (t1) and in the final product (Table 3). Furthermore, Table 2 shows that the TA in CCB2 was higher than in CCB1 in both the fermented dough and the final bread product, with generally lower pH values observed. This higher TA and lower pH contribute to the preservation of the bread by creating an acidic environment that inhibits fungal growth, which is consistent with findings reported by Katsi et al. (2021). It is important to note that lactic acid is not the only acid contributing to TA; other acids produced during fermentation also play a significant role, resulting in varying TA values across different sourdough samples.

Table 3
Determination of lactic acid levels in freshly made dough, fermented dough, and bread.

	Lactic acid (mg lactic acid/kg of the product)		
	Freshly made dough t_0	Fermented dough t_1	Bread
CB	48.50 ± 2.39^{cB}	51.03 ± 0.92^{gA}	69.54 ± 0.68^{gA}
CPB	132.46 ± 1.71^{bB}	131.74 ± 1.36^{fB}	139.97 ± 1.71^{fA}
CCB 1	149.84 ± 11.26^{bC}	247.07 ± 0.68^{eA}	202.19 ± 1.36^{eB}
CCB 2	123.44 ± 0.68^{bB}	627.54 ± 2.39^{aA}	569.88 ± 4.78^{aB}
SB 10%	77.21 ± 9.55^{cB}	91.45 ± 3.75^{hAB}	224.96 ± 2.39^{eA}
SB 20%	324.03 ± 8.53^{aB}	400.09 ± 1.02^{cA}	403.65 ± 0.34^{dA}
SB-TI6 10%	156.59 ± 14.67^{bC}	294.84 ± 1.36^{dB}	470.72 ± 1.02^{cA}
SB-TI6 20%	293.39 ± 4.09^{aC}	508.84 ± 0.34^{bB}	527.9 ± 2.73^{bA}

CB: control bread; CPB: bread control with propionate; CCB 1: commercial sourdough bread 1; CCB 2: commercial sourdough bread 2; SB 10%: sourdough bread with control sourdough inoculum (10 %); SB 20%: sourdough bread with control sourdough inoculum (20 %); SB-TI6 10%; sourdough bread with *P. pentosaceus* TI6 inoculum (10%); SB-TI6 20%; sourdough bread with *P. pentosaceus* TI6 inoculum (20%). Different lowercase letters mean a significant difference in lactic acid concentration between samples at the same time of bread making. Different capital letters mean significant differences in the concentration of lactic acid between the 3 steps of the bread elaboration ($p \leq 0.05$).

Notably, sourdough bread typically possesses a pH ranging from 3.4 to 4.9, rendering it more acidic than conventional breads (Kazakos, Mantzourani & Plessas, 2022). This pH value is influenced by several factors, such as the type of flour utilized, fermentation duration, temperature, and humidity of the environment (Casado et al., 2017). In the present study, the pH values of the sourdough breads align with those reported by Lappi et al. (2010), where a decrease in pH was observed after the fermentation process. As demonstrated, prolonged fermentation times yield slightly lower pH levels.

Overall, these findings underscore the importance of pH control in sourdough bread production, as it not only impacts the bread's organoleptic properties but also contributes to its extended shelf life.

In terms of TA, the values demonstrated an increase during the fermentation process of the doughs. This observation aligns with the findings of Katsi et al. (2021), who established a correlation between sourdough bread having lower pH and higher TA, resulting in an extended shelf life due to the inhibitory effect of the acidic environment on fungal growth.

The microbiological population of the doughs and breads is also presented in Table 2. A substantial population of lactic acid bacteria (LAB) in the dough is crucial for product safety, as LAB plays a preventive role against the proliferation of problematic microorganisms, such as fungi (Lau, Chong, Chin, Talib, & Basha, 2021).

Analysis of the total microorganisms in the freshly made doughs SB-TI6 10% and SB-TI6 20% reveals higher microbiological growth compared to the other doughs. Similarly, the CPB dough exhibits a notable content of microorganisms. Illueca et al. (2023) reported that the addition of propionate in bread-making can enhance microorganism growth. During the fermentation process, the microbiological population in the fermented doughs experiences an increase. The fermented doughs SB-TI6 10% and SB-TI6 20% demonstrate that the addition of LAB during bread elaboration can contribute to a rise in microorganism growth. Similarly, the CPB fermented dough and the CCB 1 sample also exhibit a high number of microorganisms. After baking the dough, a decrease in the total microorganism count is observed. However, in the breads SB-TI6 10%, SB-TI6 20%, CCB 1, and CCB 2, the content of microorganisms remains present, indicating that the addition of sourdough influences the microbiological population and fosters the proliferation

of LAB.

Regarding yeasts, at t0 (freshly made dough), yeast growth is only evident in control breads CB and CPB, with no detectable values in the other samples. However, after dough fermentation, the yeast population increases in all the fermented doughs, with SB-TI6 10%, SB-TI6 20%, and the control samples CPB and CCB 1 exhibiting prominent yeast growth. Finally, in the final product (bread), yeasts are no longer detected, as the high temperature reached during baking inhibits their development.

Focused on the final properties of the bread, the specific volume results are presented in Table 2. There are significant differences between the samples with sourdough addition and the other breads. Notably, the specific volume results were higher in SB-TI6 10% and SB-TI6 20% compared to the rest of the samples. The incorporation of sourdough in the bread-making process establishes an optimal pH environment for enzymatic activity, leading to increased volume. Additionally, it retards starch retrogradation and bread firming while enhancing texture and flavor, as noted by Katsi et al. (2021). The acidic medium also augments the gas retention capacity of the gluten structure due to the solubility of pentosans during sourdough fermentation, as highlighted by (Ertop & Şeker, 2018).

The a_w and moisture results are detailed in Table 2. The aw values ranged from 0.6 to 0.85. Sourdough breads exhibited lower aw values, likely attributed to the metabolic activity of LAB during fermentation. A_w serves as a measure of available free water in the product and is crucial in defining food quality and safety, particularly concerning microbial growth and physical and chemical attributes, as emphasized by De Luca et al. (2021). A lower a_w value inhibits fungal development, making products with lower water activity less conducive to microbiological growth. Among the breads, the sourdough variant SB recorded the lowest aw values. On the other hand, Debonne et al. (2020) study shows that the a_w values of both par-baked and full-baked bread crumbs were not affected by the concentration of sourdough while baking parameters such as baking time and temperature can impact a_w and consequently the shelf life of bread.

The fermentation step in bread making typically reduces the Aw of the bread. During fermentation, yeast and bacteria metabolize sugars and produce metabolites like ethanol and lactic acid. This process not only changes the chemical composition of the dough but also binds some of the water, making it less available for microbial activity. As a result, the Aw decreases, which can contribute to the stabilization and preservation of the bread by inhibiting the growth of spoilage organisms and extending its shelf life (Akamine, Mansoldo, & Vermelho, 2023).

In terms of moisture content, there were no significant differences observed among the samples; the moisture content remained consistent at approximately 30–31 g/100 g in all breads. This moisture range is in line with findings by Hendek Ertop et al., 2018, who reported higher humidity levels in bread containing dried sourdough. They underscored that moisture retention is a critical factor in the product's shelf life, as moisture loss is closely related to staling properties. Ertop and Şeker (2018) also demonstrated that the use of sourdough in bread production leads to reduced moisture loss compared to control bread made with bakery yeast. Similar results were found in Hendek Ertop et al. (2018) study, indicating that bread prepared with oven-dried sourdough experiences a gradual moisture loss between the second and fourth day, contrasting with the rapid moisture loss observed in the control sample.

3.3. The content of lactic acid in dough and bread

LAB are microorganisms capable of using different substrates to produce organic acids through the fermentation process, including carbohydrates (Abedi & Hashemi, 2020). Lactic acid is the main organic acid produced by LAB and it is responsible for some characteristics and properties of many fermented foods, including sourdough bread (Akamine et al., 2023).

The data of the lactic acid determined in the samples at the three times of bread elaboration; freshly made dough (t_0), fermented dough (t_1), and bread are plotted in Table 3. The lactic acid results presented significant differences between the samples and the times. It can be observed that the dough at t_0 shows lower values of lactic acid, especially the control treatment, CB. After fermentation, lactic acid production augmented in all the samples except for the control treatments, CB, and CCB which showed the lowest results of the samples. Finally, in bread, the lactic acid content increased due to the evaporation of the water during baking which resulted in a major concentration of this organic acid in the final product. Highlights the highest content of lactic acid in CCB 2 and SB-TI6 20% samples.

Results in Table 3 evidenced that the use of dried sourdough in breadmaking results in higher production of lactic acid. So, the use of the dried powder enhanced the production of this organic acid. However, the sourdough fermented with *P. pentosaceus* TI6 shows significant differences compared to the sourdough without LAB inoculum. Moreover, it can be seen the increase in lactic acid through the 3 steps of the bread elaboration.

Firstly, the selection of an LAB strain with antifungal activity is an important step to allow the elaboration of a great product with improved food safety, as can be seen in the previous study by Lafuente et al. (2023). Related to the antifungal activity of the strain *P. pentosaceus*, which showed an inhibitory effect against some fungi. The use of LAB has an important effect on sourdough elaboration due to its activity. LAB plays an important role in the production of many fermented foods, and their ability to produce organic acids, mainly lactic acid, contributes to the special flavors and textures of these products.

Boyaci Gunduz et al. (2022) study found that the selection of LAB strains has an impact on the chemical and microbiological characteristics of sourdough during the fermentation process. The study shows *L. plantarum* microorganism as the most efficient LAB strain to produce sourdough with high lactic acid content and the lowest pH.

In the case of the dried fermented sourdough by *P. pentosaceus* used in the preparation of bread, it improves the properties of the final product and lengthens its useful life due to the acidified environment due to the metabolism of the LAB that produces antifungal compounds. Thus, the employment of LAB brings benefits in food biopreservation since produces compounds with activity against fungi, such as organic acids among others and the unfavorable conditions of the acidified medium hinder the fungal growth (Nasrollahzadeh, Mokhtari, Khomeiri, & Saris, 2022). The lactic acid passes through the membrane to reach the fungi and break down the cells, which leads to the release of hydrogen ions and results in a decrease in pH. And, to enter the cell, organic acids need to be converted into an undissociated form (Jung, Hwang & Lee, 2019).

3.4. Evaluation of the antifungal activity of the breads

The reduced shelf life of bread can be attributed to both aging and mold. The performance of the samples in the antifungal activity evaluation is detailed in Table 4. The study on the shelf life of the loaves demonstrated a similar trend against two inoculated fungi, *A. flavus* and *P. verrucosum*. The inclusion of dried sourdough in the production process extended the product's shelf life by up to 4 days compared to the other samples, with the most significant effect observed in SB-TI6 20%. Additionally, the incorporation of calcium propionate in CPB bread effectively prevented fungal growth, sustaining product integrity until the final day of the assessment.

Interestingly, bread loaves contaminated with *A. flavus* exhibited a comparable shelf life when compared to those contaminated with *P. verrucosum*. In both instances, visible fungal growth in the control bread (CB) and commercial products (CCB 1 and CCB 2) commenced on the second day. However, in the case of bread inoculated with A. flavus, the presence of dried sourdough delayed fungal development by one day for SB 10% and SB 20%, and by days 4 and 5 for SB-TI6 10% and SB-TI6 20%, respectively. Similarly, in the bread inoculated with *P. verrucosum*,

Table 4
Qualitative results of the shelf-life study of the different bread samples inoculated with spores of *Aspergillus flavus* and *Penicillium verrucosum*.

Bread	Fungi	Shelf life						
		1	2	3	4	5	6	7
CB	*A. flavus*	−	+	+	+	+	+	+
CPB		−	−	−	−	−	−	+
CCB 1		−	+	+	+	+	+	+
CCB 2		−	+	+	+	+	+	+
SB 10%		−	−	+	+	+	+	+
SB 20%		−	−	+	+	+	+	+
SB-TI6 10%		−	−	−	+	+	+	+
SB-TI6 20%		−	−	−	−	+	+	+
CB	*P. verrucosum*	−	+	+	+	+	+	+
CPB		−	−	−	−	−	−	+
CCB 1		−	+	+	+	+	+	+
CCB 2		−	+	+	+	+	+	+
SB 10%		−	−	−	+	+	+	+
SB 20%		−	−	−	+	+	+	+
SB-TI6 10%		−	−	−	+	+	+	+
SB-TI6 20%		−	−	−	+	+	+	+

CB: control bread; CPB: bread control with propionate; CCB 1: commercial sourdough bread 1; CCB 2: commercial sourdough bread 2; SB 10%: sourdough bread with control sourdough inoculum (10 %); SB 20%: sourdough bread with control sourdough inoculum (20 %); SB−TI6 10%; sourdough bread with *P. pentosaceus* TI6 inoculum (10%); SB−TI6 20%; sourdough bread with *P. pentosaceus* TI6 inoculum (20%).

all four samples containing dried sourdough prevented fungal appearance until the fourth day. Consequently, the utilization of the product fermented by *P. pentosaceus* TI6 effectively curtails fungal contamination in bread. Moreover, the application of oven-dried sourdough serves as a functional powder, given that bread consumption primarily occurs within the first few days after production.

Regarding the fungal population in the bread loaves contaminated with *A. flavus* and *P. verrucosum*, data were collected during the shelf-life assessment, and the results are presented in Fig. 3. In the case of loaves inoculated with *A. flavus*, the highest microbial counts were observed in the control bread (CB), as well as the commercial products CCB 1 and CCB 2, and the sourdough bread SB 10%, with values ranging from 6.5 to 7 log CFU/g. Conversely, in the breads contaminated with *P. verrucosum*, a lower number of fungi were observed compared to the *A. flavus* contamination. However, the same samples exhibited the highest fungal counts, ranging from 6 to 6.5 log CFU/g. The inclusion of dried sourdough fermented with *P. pentosaceus* in the bread formulation effectively inhibited fungal growth. In both microbiological analyses, the SB-TI6 20% sample demonstrated the lowest fungal viability. Additionally, the addition of propionate contributed to a reduction in the microbial population.

Numerous studies have corroborated these findings, highlighting the extended shelf life of bread when sourdough fermented by lactic acid bacteria (LAB) is incorporated, effectively reducing fungal growth (Ertop, Ilter, YilMaz, BaltaCi, & Gündogdu, 2018).

The utilization of sourdough at both 10% and 20% levels in bread production has proven to be effective in evaluating the product's antifungal capacity against contamination. However, it's conceivable that an even higher percentage may yield more favorable results in terms of inhibiting fungal growth. This approach could potentially lead to an extended shelf life, as demonstrated by the improved performance observed when using 20% of oven-dried sourdough, which resulted in a reduced fungal population.

Further experimentation with higher sourdough percentages would be a valuable avenue for research to assess the full potential of sourdough as an antifungal agent in bread production. Such investigations could provide additional insights into the optimal sourdough concentration for maximizing shelf life and minimizing fungal contamination.

3.5. Mycotoxins analysis of the breads

Some mycotoxins, such as aflatoxins (AF), ochratoxins (OTA), patulin, fumonisins, citrinin, zearalenone, and trichothecenes are important mycotoxins that have been detected in several investigations. The mycotoxins production affects both producers and consumers concerning health and effectiveness costs. Cereals and cereal-based products are some of the products most susceptible to mycotoxin contamination. Some fungi, such as *A. flavus* and *P.verrucosum* are mycotoxigenic contaminating crops throughout pre and post-harvest periods (El-Sayed, Jebur, Kang, & El-Demerdash, 2022). Despite the

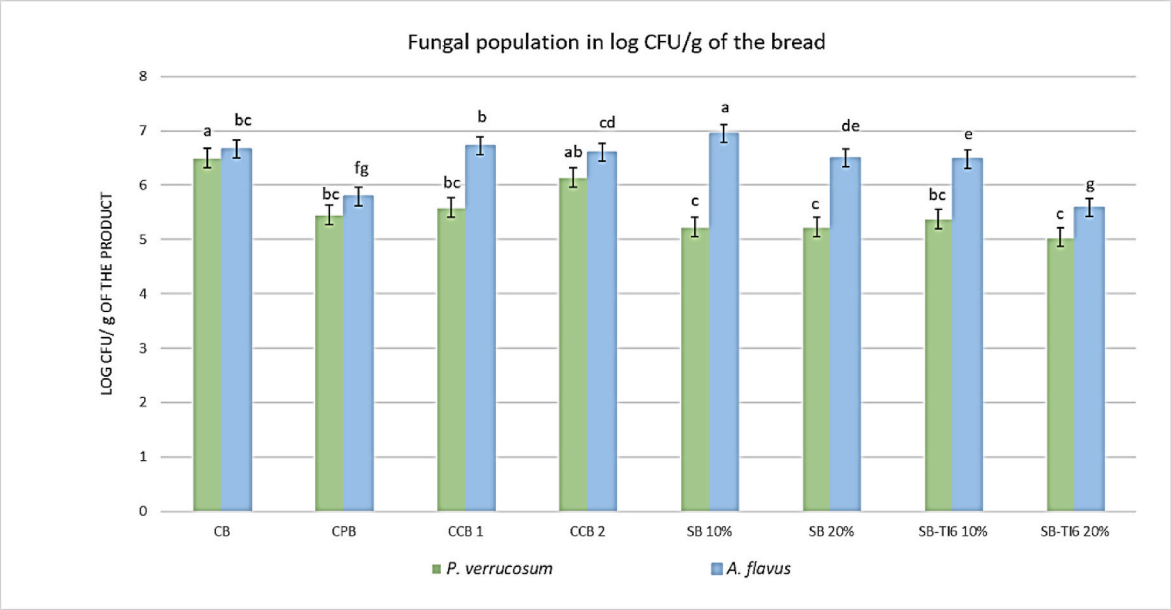

Fig. 3. Fungal population in log CFU/g of bread contaminated with *A. flavus* and *P. verrucosum*. CB: control bread; CPB: bread control with propionate; CCB 1: commercial sourdough bread 1; CCB 2: commercial sourdough bread 2; SB 10%: sourdough bread with control sourdough inoculum (10%); SB 20%: sourdough bread with control sourdough inoculum (20%); SB−TI6 10%; sourdough bread with *P. pentosaceus* TI6 inoculum (10%); SB−TI6 20%; sourdough bread with *P. pentosaceus* TI6 inoculum (20%). Different lower−case letters indicate significant differences between samples ($p \geq 0.05$).

good agricultural practices to reduce mycotoxin exposure, some contamination may develop during manufacturing or storage steps.

The mycotoxins determination in breads can be observed in Table 5, and the results were obtained in relation to the shelf-life study. The presence of aflatoxins (AF) was detected in breads contaminated by *A. flavus*. And there was no detection of ochratoxin A (OTA) in the breads contaminated by *P. verrucosum*, as shown in the study of Illueca et al. (2023).

Highlight the lower levels of AF mycotoxins in bread with sourdough fermented with *P. pentosaceus* TI6. Table 5 plots the mycotoxin levels of the loaves contaminated with *A. flavus*. The results showed the highest levels of AFB1 production, which reached values of 330 mg of AFB1 per Kg of product. Control bread loaves CB and the commercial product CBB 1 show the highest levels of mycotoxin contamination. The SB−TI6 20% contaminated loaves reduced the concentration of AFs, being the addition of the dry.

The fermentation sourdough process of LAB *P. pentosaceus* and its use in sourdough bread elaboration evidenced a mycotoxin reduction. LAB grows naturally in fermented products and can be used as biocontrol microorganisms to decrease the mycotoxin values of contaminated food (Badji et al., 2023). Several studies have investigated the reduction of mycotoxins using LAB to ferment food, specifically the use of sourdough to reduce mycotoxins in breads. Hassan, Zhou, and Bullerman (2016) showed that the action of sourdough LAB in mycotoxin reduction can be classified generally under two modes of action: mycotoxin biodegradation/bioconversion, consisting of microorganisms that can reshape and metabolize mycotoxins, and mycotoxin binding, another potential method that by binding to bacteria present in the normal gut flora or in fermented products, the uptake of mycotoxins is blocked.

Fazeli et al. (2009) investigated the reduction of mycotoxins by the anticarcinogenic activity of LAB isolated from Iranian bread. The study shows that the strains *L. plantarum*, *L. fermentum,* and *L. casei* evidenced significant activity in the reduction of aflatoxins in liquid media.

The choice of *P. pentosaceus*, a homofermentative strain, for sourdough fermentation in our study is justified by its superior antifungal properties and comprehensive benefits for bread quality and safety. Although acetic acid is traditionally favored for extending bread shelf-life, *P. pentosaceus* TI6 demonstrated remarkable antifungal capacity, producing significant levels of VOCs such as benzeneacetic acid and pyrazines. These compounds contributed to inhibiting fungal growth and enhancing microbial stability. Additionally, the strain's production of a variety of organic acids and phenolic compounds, including phenyllactic acid, offered synergistic effects that extend beyond the capabilities of acetic acid alone. Therefore, *P. pentosaceus* TI6 not only improved the safety and shelf-life of sourdough bread but also enhanced its flavor and overall quality, making it a superior choice for fermentation (Lafuente et al., 2023).

When considering the safe use of *P. pentosaceus* in food, particularly for bread, several studies consider the potential advantages and safety concerns of *P. pentosaceus*. Indeed, the experimental findings discussed that some *P. pentosaceus* strains showed high anti-microbial activity which could increase the quality and safety of bread through the suppression of fungi considered one of the spoilage-causing microorganisms (Bartkiene et al., 2022). It was revealed that *P. pentosaceus* included LAB is a promisor strain with good inhibitory capacity with the lactic acid which showed several potential advantages in construction: anti-inflammation, anticancer, antioxidant, detoxification, and lipid-lowering properties, and qualification for use as an excellent probiotics' candidate (Jiang, Cai, Lv, & Li, 2021).

Furthermore, safety assessments should be performed for the screened *P. pentosaceus* strains to ensure their suitability for food processing (Jiang et al., 2021). Studies have demonstrated that *P. pentosaceus* strains can enhance the fermentation process in bread, leading to improved texture, flavor, and quality parameters, while also showing resistance to mold and rope spoilage, which are crucial factors in ensuring the safety and shelf-life of bread products (Qi et al., 2021).

In particular, *P. pentosaceus* is recognized for its broad application in food processing due to its numerous probiotic benefits and its classification as a safe, food-grade microorganism. The safety of *P. pentosaceus* in food products has been established through extensive studies that demonstrate its non-toxicity and absence of pathogenicity. *In vitro* safety assessments have covered aspects such as the lack of hemolytic activity, antibiotic resistance, and toxin production, ensuring it does not pose health risks when consumed. Furthermore, *P. pentosaceus* has been shown to exhibit significant antioxidant, cholesterol-lowering, and immune-modulating effects, contributing to overall health benefits. This strain has been isolated from a variety of fermented foods, suggesting its inherent stability and safety in diverse food matrices. Additionally, *P. pentosaceus* has been effectively used in dairy, vegetable, and meat fermentations, enhancing the sensory qualities and safety of the final products. The extensive research supporting the safety and probiotic potential of *P. pentosaceus* makes it a reliable choice for its use in the food industry(Qi et al., 2021).

Regarding the disadvantages of our study, we can emphasize that our results provide valuable insights into the antifungal and antitoxigenic properties of breads made with lacto-fermented dried sourdough, but some limitations should be considered. Firstly, this work was restricted to *the P. pentosaceus* strain and, this constraint may limit the generalizability of the results across different types of sourdough and microbial strains. Secondly, our experimental design focused only on a short-term analysis of bread shelf life and mycotoxin levels. Longer-term studies might reveal additional insights into the stability and efficacy of the antifungal properties over extended periods although we have ceased the shelf life experiment when the fungal growth was evident. Additionally, the study did not address the sensory impact of using dried sourdough in bread production, which is crucial for consumer acceptance. Therefore, we suggest that future studies should consider a broader range of LAB strains, extend the duration of shelf life studies, and include sensory evaluation to better understand the practical applications and consumer perceptions of these breads.

Table 5
Production of Aflatoxins (AFB1, AFB2, AFG1, AFG2) in breads contaminated with *Aspergillus flavus*.

Samples	Mycotoxins (mg mycotoxin/Kg of the bread contaminated with *A. flavus*)			
	AFB1/ Aflatoxin B1	AFB2/ Aflatoxin B2	AFG1/ Aflatoxin G1	AFG2/ Aflatoxin G2
CB	0.232 ± 0.006 ^{b}	0.016 ± 0.001 ^{b}	0.004 ± 0.002 ^{ab}	0.005 ± 0.001 ^{bc}
CPB	0.012 ± 0.001 ^{d}	0.001 ± 0.001 ^{d}	0.000 ± 0.000 ^{b}	0.000 ± 0.001 ^{c}
CCB 1	0.331 ± 0.046 ^{a}	0.029 ± 0.002 ^{a}	0.006 ± 0.001 ^{a}	0.007 ± 0.001 ^{a}
CCB 2	0.144 ± 0.004 ^{c}	0.005 ± 0.003 ^{cd}	0.000 ± 0.000 ^{b}	0.007 ± 0.001 ^{a}
SB 10%	0.250 ± 0.033 ^{b}	0.009 ± 0.002 ^{c}	0.004 ± 0.003 ^{ab}	0.006 ± 0.001 ^{b}
SB 20%	0.190 ± 0.014 ^{bc}	0.004 ± 0.001 ^{cd}	0.004 ± 0.001 ^{ab}	0.005 ± 0.001 ^{bc}
SB−TI6 10%	0.097 ± 0.007 ^{c}	0.005 ± 0.003 ^{cd}	0.002 ± 0.001^{b}	0.006 ± 0.001 ^{b}
SB−TI6 20%	0.024 ± 0.062 ^{d}	0.003 ± 0.002 ^{cd}	0.001 ± 0.001 ^{b}	0.004 ± 0.001 ^{bc}

CB: control bread; CPB: bread control with propionate; CCB 1: commercial sourdough bread 1; CCB 2: commercial sourdough bread 2; SB 10%: sourdough bread with control sourdough inoculum (10%); SB 20%: sourdough bread with control sourdough inoculum (20%); SB−TI6 10%; sourdough bread with *P. pentosaceus* TI6 inoculum (10%); SB−TI6 20%; sourdough bread with *P. pentosaceus* TI6 inoculum (20%). Different lower−case letters indicate significant differences between samples ($p \geq 0.05$).

4. Conclusions

Advances in microbiology have made commercially available bread common, relying on selected yeasts and artificial preservatives. However, sourdough's benefits, with its rich microbiome, are gaining recognition. Drying techniques are being explored to facilitate sourdough's industrial use. The main objectives of this study were to use dried sourdough fermented by *P. pentosaceus* TI6 in the elaboration of bread, evaluate the properties of the product, and determine the antifungal activity of the dried product in the bread. The effect of the volatile profile of the dried sourdoughs positively affects the properties of the product so that the oven−dried sourdough powder with more antifungal compounds. This study found significant differences in bread when using dried sourdough compared to control and commercial bread. The dried sourdough resulted in a more acidic medium, enhancing product volume through improved enzyme action and gas retention. This acidity also led to higher organic acid content, reaching 520 mg of lactic acid per kg of product. On the other hand, reduced water activity in sourdough bread prevented fungal contamination and undesirable microorganism growth, extending shelf life, and reducing mycotoxin levels. Accordingly, the use of dry sourdough fermented by LAB *P. pentosaceus* TI6 proved effective in enhancing bread quality and safety.

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CRediT authorship contribution statement

Carla Lafuente: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Tiago de Melo Nazareth:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Victor Dopazo:** Writing – original draft, Methodology, Formal analysis, Data curation. **Giuseppe Meca:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization. **Carlos Luz:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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