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Effects of Various Inocula Combinations of *Leuconostoc mesenteroides*, *Papiliotrema terrestris*, and *Saccharomyces cerevisiae* on Dough Fermentation and Final Bread Characteristics

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Abstract: The integration of yeast and lactic acid bacteria (LAB) in bakery products is currently trending, which aims to enhance the taste and quality to satisfy consumer preferences. This study explored the interaction of *Leuconostoc mesenteroides*, *Saccharomyces cerevisiae*, and *Papiliotrema terrestris* during dough fermentation. Yeasts and LAB were monitored every three hours over a twelve-hour period. The chemical parameters and quality characteristics of both the dough and bread were analyzed. The highest level of *S. cerevisiae* was observed in the control treatment (9.30 log CFU/g, after 9 h) and in the treatment with co-inoculation of *S. cerevisiae* and *P. terrestris* (9.30 log CFU/g, after 12 h). Non-*Saccharomyces* yeasts peaked in the treatment with *P. terrestris* and *L. mesenteroides*, showing 7.77 log CFU/g after three hours. Doughs with *L. mesenteroides* achieved the fastest acidification. Treatments involving all three strains showed the highest increase in volume and CO_2 emissions. The profiles of volatile organic compounds emitted from bread varied depending on the inoculum combination. These findings underscore the need for further research into the interactions between this unconventional yeast and other microorganisms typically used in baking.

Keywords: fermentation; breadmaking; yeasts; lactic acid bacteria; non-*Saccharomyces* yeasts; *Saccharomyces cerevisiae*; *Leuconostoc mesenteroides*; *Papiliotrema terrestris*

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1. Introduction

Bread is predominantly produced through biological leavening, involving either yeasts or sourdough technology [1]. Among yeasts in the *Saccharomyces* genus, *Saccharomyces cerevisiae* is the most widely used in breadmaking. This species enables controlled fermentations, where the microbial agent (starter culture) is selected in the laboratory and added to raw materials to expedite fermentation and enhance product quality and safety. Due to its characteristics such as flavor production, lack of toxin production, and high ethanol output, *Saccharomyces cerevisiae* is extensively used in the industrial fermentation of foods, such as bread, beer, and wine [2]. The use of selected *S. cerevisiae* strains has reduced fermentation duration and standardized the overall quality but it has also decreased the sensory complexity of the final product [3]. On the other hand, sourdough fermentation is a biotechnological process that improves the properties of bakery products, including

bread. During dough fermentation, yeasts and lactic acid bacteria (LAB) interact, utilizing available nutrients to enhance the functional and nutritional properties of the bread [4].

Selected starter yeasts and LAB play a key role in determining bread quality. Hu et al. [5] investigated the interaction between yeasts and LAB by comparing control production using *S. cerevisiae* Y338 with combinations involving *Lactiplantibacillus plantarum* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, either individually or together. The study found that the presence of *L. plantarum* led to better results in terms of the volume increase, volatile organic compounds (VOCs), and bread softness; however, these findings cannot be generalized, as product improvement conditions are closely related to the specific starter strains, which can trigger a variety of complex biochemical reactions. Recently, the growing interest in traditional products and the quest for unique sensory characteristics has drawn academic attention to the potential use of non-conventional yeasts in breadmaking [6]. While *S. cerevisiae* remains the predominant yeast in breadmaking, its ability to utilize sugars in cereal flours to produce CO_2, essential for volume increase, is not exclusive to this species as once thought. Recent studies have highlighted the benefits of using non-conventional, or non-*Saccharomyces*, yeasts to improve bakery products, particularly their aromatic attributes [7]. Nonetheless, the challenge with non-*Saccharomyces* yeasts often lies in their fermentation performance, which may not match that of *Saccharomyces* yeasts [8].

The use of non-conventional yeasts presents a promising avenue for further improving bakery products in the near future. Wittwer and Howell [9] discussed the potential use of non-conventional yeasts (*Kazachstania bulderi*, *Kazachstania gamospora*, *Kluyveromyces marxianus*, *Lachancea fermentati*, *Saccharomyces bayanus*, *Torulaspora delbrueckii*, *Wickerhamomyces anomalus*, and *Wickerhamomyces subpelliculosus*) in breadmaking. They emphasized that several issues related to industrial applicability still require further investigation. Currently, there is insufficient knowledge about the metabolic requirements of non-conventional yeasts, and it remains unclear whether their fermentation performance is superior in co-culture or in combination with certain substrates or enzymes beyond laboratory settings. In the near future, combining yeasts with LAB either in co-culture or as a single inoculation will become a primary goal to enhance bakery product quality and meet current market demands. Consumers are becoming increasingly aware of health and quality aspects.

The *Papiliotrema terrestris* strain PT22AV—active ingredient in the water-dispersible granule formulation marketed as YSY® by AgroVentures LLC and srl (Latina, Italy)—is renowned for its effectiveness in controlling numerous plant diseases both in the field and post-harvest. Formulations based on strain PT22AV have been particularly successful against nematode [10] necrotrophic fungal pathogens, including those from the genera *Monilia*, *Botrytis*, *Penicillium*, *Fusarium*, *Rhizoctonia*, and *Pythium* [11]. Furthermore, strain PT22AV is known for producing exopolysaccharides, extracellular metabolites with high functional value and nutraceutical, therapeutic, and industrial potential [12]. Hamidi et al. [13] explored the antibacterial and antioxidant properties of exopolysaccharides produced by strain PT22AV, highlighting their potential as biopolymers in medical product manufacturing. In the food industry, exopolysaccharides are primarily used as thickening agents [14]. Extensive research on microbial-origin exopolysaccharides in bakery products has shown these additives to enhance dough viscosity [15], reduce bread firmness [16], and increase bread specific volume [17] and crumb softness [18].

Research on the use of *P. terrestris* in breadmaking is limited. A preliminary study on the microbial ecology of yeasts isolated from various durum wheat varieties [19] reported that co-inoculating the *P. terrestris* strain D12 with a commercial strain of *S. cerevisiae* resulted in reduced dough weight and CO_2 produced after 24 h, suggesting its potential to enhance bread sensory profiles. To leverage this non-*Saccharomyces* yeast (*P. terrestris*) for improving the aromatic characteristics of bread, different microorganism combinations were investigated to produce different doughs. This study evaluated the interactions between *P. terrestris* PT22AV and *S. cerevisiae* and/or *Leuconostoc mesenteroides*, both individually and in combination. This research contributes to expanding and deepening

the understanding of alternative dough fermentation methods, ultimately advancing the production of baked goods.

2. Materials and Methods

2.1. Starter Strains

The fermentation process was carried out with the following microorganisms: *Saccharomyces cerevisiae* [~4.00 × 10^9 CFU/g], available as active dry yeast by Conad S.C. (Bologna, Italy); YSY® [*Papiliotrema terrestris* PT22AV (~3.50 × 10^9 CFU/g)], freeze-dried and provided by AgroVentures srl (Latina, Italy); and Lyoflora BLN-1 [*Leuconostoc mesenteroides* (~1.00 × 10^{11} CFU/g)], freeze-dried from Sacco srl (Cadorago, Italy). YSY® and Lyoflora BLN-1 were kindly donated by Bruno Folchi, General Manager at AgroVentures srl, while the *S. cerevisiae* was purchased from a local Conad supermarket (Palermo, Italy).

2.2. Strain Rehydration

The rehydration of the three strains was performed using sterile mineral water (Acqua Vera spa, Milan, Italy) by adding 31.25 mL of water to 3.125 g of each liophilized strain. The suspension was left in static conditions at 37 ± 1 °C for 10 min in a thermostatic water bath TRM 740 (Asal srl, Cernusco, Italy).

2.3. Experimental Design, Dough Production, and Baking

The experimental plan, illustrated in Figure 1, aimed to evaluate five different types of dough: C1, control dough inoculated with *Leuconostoc mesenteroides*; C2, control dough inoculated with *S. cerevisiae*; B1, experimental dough co-inoculated with *L. mesenteroides* and *Papiliotrema terrestris*; B2, experimental dough co-inoculated with *S. cerevisiae* and *P. terrestris*; B3, experimental dough co-inoculated with *L. mesenteroides*, *P. terrestris,* and *S. cerevisiae*.

Doughs (500 g each) were produced with a dough yield (DY, weight of the dough/weight of flour × 100) of 160, following the recipe reported in Table 1. The ingredients included type-0 flour (Molino F.lli Chiavazza spa, Casalgrasso, Italy), bottled water (Acqua Vera spa, Milan, Italy), and kitchen salt (Sosalt, Trapani, Italy) [19].

Table 1. Dough recipe.

Ingredients	Treatments				
	C1	**C2**	**B1**	**B2**	**B3**
Mineral water (mL)	156.25	156.25	125	125	93.75
Flour type 0 (g)	312.5	312.5	312.5	312.5	312.5
Kitchen salt (g)	10	10	10	10	10
Baker's yeast inoculum (mL)	0	31.25	0	31.25	31.25
Lyofora BLN-1 inoculum (mL)	31.25	0	31.25	0	31.25
YSY® inoculum (mL)	0	0	31.25	31.25	31.25

Abbreviations: C1, control trial inoculated with *Leuconostoc mesenteroides*; C2, control trial inoculated with *Saccharomyces cerevisiae*; B1, trial co-inoculated with *L. mesenteroides* and *Papiliotrema terrestris*; B2, trial co-inoculated with *S. cerevisiae* and *P. terrestris*; B3, trial co-inoculated with *L. mesenteroides*, *P. terrestris*, and *S. cerevisiae*.

All ingredients were mechanically mixed using a planetary mixer model XBM10S (Electrolux Professional spa, Pordenone, Italy) equipped with a spiral paddle. The process consisted of two steps: the first at speed 1 (45 rpm) for 5 min, followed by the second at speed 2 (90 rpm) for 2 min at room temperature. Bread production was performed in duplicate (two technical repeats) and repeated twice (two independent replicates) after 2 weeks.

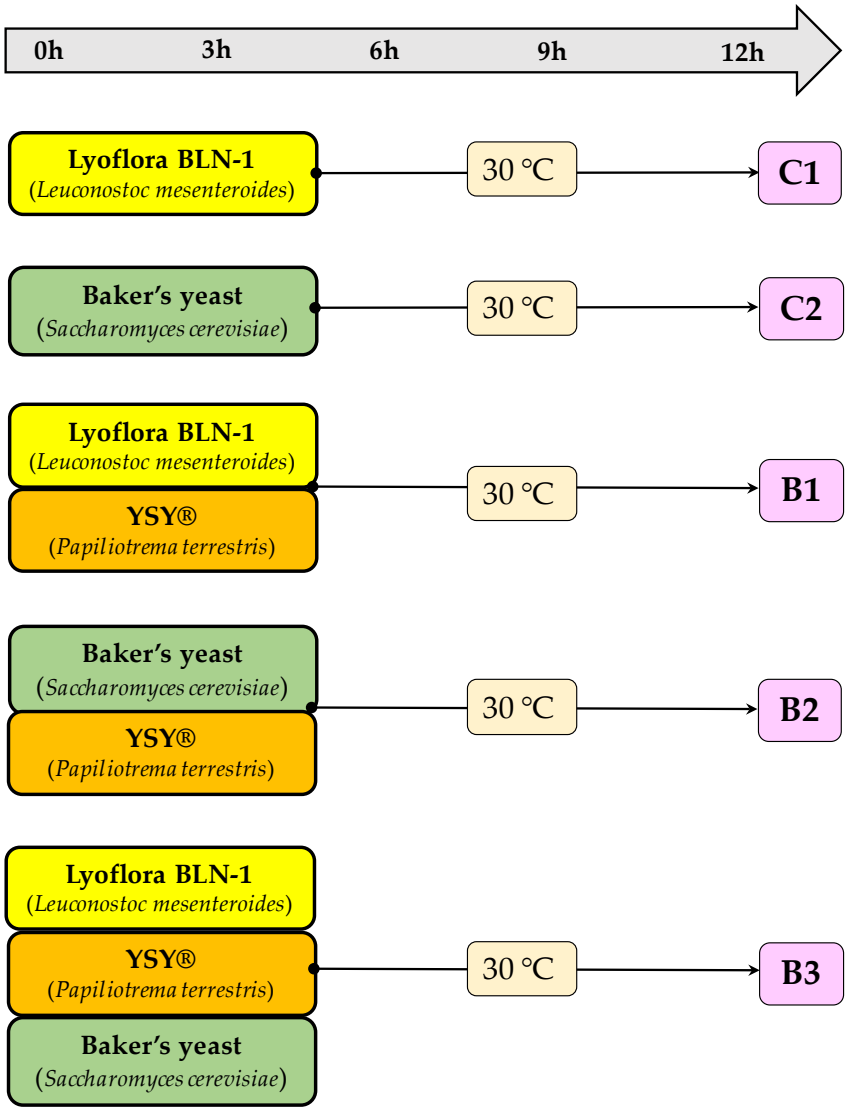

Figure 1. Experimental design of dough production. Abbreviations: C1, control trial inoculated with *Leuconostoc mesenteroides*; C2, control trial inoculated with *Saccharomyces cerevisiae*; B1, trial co-inoculated with *L. mesenteroides* and *Papiliotrema terrestris*; B2, trial co-inoculated with *S. cerevisiae* and *P. terrestris*; B3, trial co-inoculated with *L. mesenteroides*, *P. terrestris*, and *S. cerevisiae*.

Three 100-gram dough samples per trial were placed into stainless steel baking pans with trapezoidal dimensions [143 × 79 mm (top inside), 129 × 64 mm (bottom outside), 57 mm (depth inside)] as recommended by the American Association of Cereal Chemists [20].

The remaining 200 g of dough was placed into a sterile polypropylene container (MedicalMarket, Roma, Italia) for microbiological, physicochemical, and qualitative analyses. The fermentation process for all doughs was conducted at 30 ± 1 °C for 12 h in an ICN35 incubator (Argolab, Carpi, Italy). Microbiological and physicochemical parameters were monitored immediately after mixing the ingredients (0 h) and at 3, 6, 9, and 12 h of fermentation. Bread baking was carried out in a semi-industrial oven Compact Combi (Electrolux, Pordenone, Italy) at 200 °C for 5 min under hot air, followed by 15 min of convection heating at 200 °C.

2.4. Fermentation Process Monitoring

Microbiological analyses were conducted on 10 g aliquots of each dough at 0, 3, 6, 9, and 12 h from the start of fermentation. Each sample was collected under sterile conditions

and suspended in 90 mL of Ringer's solution (Sigma-Aldrich, Milan, Italy). The samples were then homogenized at maximum speed (10 strokes/s) for 2 min using a BagMixer® 400 stomacher (Interscience, Saint Nom, France).

Cell suspensions were subsequently prepared for the plate counts by performing decimal serial dilutions in sterile glass tubes, which were then inoculated into Petri plates as follows: (i) plate count agar (PCA) was used to enumerate total mesophilic microorganisms (TMM) and incubated aerobically at 30 °C for 72 h; (ii) modified de Man, Rogosa, and Sharpe (mMRS) agar to enumerate lactic acid bacteria (LAB) rods, incubated anaerobically at 30 °C for 48 h [21]; (iii) M17 agar to detect LAB cocci, incubated anaerobically at 30 °C for 48 h; (iv) sourdough bacteria (SDB) agar to count sourdough LAB, incubated anaerobically at 30 °C for 48 h. For the yeast analysis, both *Saccharomyces* and non-*Saccharomyces* yeasts were counted by plating on Wallerstein Laboratory (WL) nutrient agar incubated aerobically at 28 °C for 72 h; this medium allowed for the differentiation and classification of the yeasts into the two aforementioned groups, as reported by Alfonzo et al. [19]. Anaerobic conditions were created using hermetically sealed jars (Oxoid, Milan, Italy) and the AnaeroGen AN25 system (Oxoid, Milan, Italy). The media (mMRS, M17, and SDB) used to count the three types of LAB were added with cicloheximide (10 mg/mL) in order to inhibit yeast and mold growth, while WL agar was added with chloramphenicol (0.1 mg/mL) to avoid bacterial growth. Microbiological analyses were performed in triplicate and the final results were expressed as Log colony forming units (CFU)/g. All media and reagents were purchased from Condalab (Torrejón de Ardoz, Spain). The chemical monitoring of the doughs was realized through the measurements of pH and total titrable acidity (TTA). pH was measured by directly immersing a pH meter model HI98165 (Hanna Instruments, Ronchi di Villafranca Padovana, Italy) into 10 g of dough collected aseptically. TTA (expressed as mL of NaOH of 0.1 N/10 g of dough) was determined in the same samples used to measure pH, which were added with sterile distilled water, transferred into stomacher bags, and homogenized for 2 min at the highest speed. Both pH and TTA measurements were performed in triplicate at 0, 3, 6, 9, and 12 h after the inocula.

Furthermore, both dough volume increase and CO_2 production were monitored throughout the fermentation process. Dough volume increase [22] was measured every 3 h up to the 12th hour by measuring the height of the dough placed in sterile polypropylene containers (MedicalMarket, Rome, Italy) at 30 °C. The amount of CO_2 produced was indirectly evaluated by recording weight loss at different intervals using the same containers with perforated lids to allow CO_2 to escape during fermentation [23].

2.5. Quality of Breads

After baking, all breads were cooled at room temperature (20 ± 2 °C) for 2 h before performing weight loss, color, specific volume, and image analyses.

2.5.1. Bread Weight Loss

Weight loss was calculated using the following formula: [(weight of dough (g) − weight of bread (g))/weight of dough (g)] × 100. Analyses were performed in triplicate, and results were expressed as percentage (%).

2.5.2. Crust and Crumb Color

Bread color was assessed using Hunter's parameters (L*, a*, b*) with the Chroma Meter CR-400 (Minolta, Osaka, Japan). Furthermore, ΔL was assessed as the difference in lightness between the sample and a white standard measured on a white tile, as indicated by Hunter Associates Laboratory, Inc. (Reston, VA, USA). Color determination was carried out on both the crust (at three different spots) and the crumb (at three spots).

2.5.3. Specific Volume

The breads were measured for volume using a volumeter for bakery products (ErreCi s.r.l., Merate, Italy) according to the rapeseed substitution method of the American Association of Cereal Chemists, Method 55–50.01 [20].

2.5.4. Image Analysis

The central slice of each bread was then subjected to image analysis using an Epson scanner (Epson Perfection 4180 Photo, Seiko Epson Corp., Tokyo, Japan) at a resolution of 300 dpi. All images (TIFF format) were processed with ImageJ 1.48v software (National Institutes Health, Bethesda, MD, USA). Each image (size: 207 × 207 pixels), after being converted to greyscale (8 bits), was transformed into a binary image using the Otsu method. The following parameters were determined: (i) void fraction of total slice area (%); (ii) cell density (number of cells/cm^2); (iii) mean cell area (in mm^2).

2.6. Volatile Organic Composition

The analysis of volatile compounds was performed using gas chromatography/mass spectrometry (GC/MS) following solid-phase micro-extraction (SPME). The SPME fiber (divinylbenzene/carboxen/polydimethylsiloxane, DVB/CAR/PDMS, 50 µm, Supelco) was utilized to adsorb the volatile compounds emitted from 5 g of each bread sample kept at 40.0 ± 0.1 °C. The samples were exposed to the fiber for 60 min at 25 °C, followed by 40 min. Subsequently, the fiber was exposed to the GC inlet for 10 min for thermal desorption at 250 °C. The temperature program for GC was set from 40 to 230 °C at a rate of 4 °C/min, with an isothermal hold for 40 min. A DB-624 capillary column (Agilent Technologies, 60 m, 0.25 mm, 1.40 µm) was employed for chromatographic analysis. The GC–MS instrument operated at 70 eV in the EI mode, scanning the m/z range from 30 to 550. Helium was used as the carrier gas at a flow rate of 1 mL/min. Compound identification was achieved by comparing the fragmentation patterns of the experimental mass spectra with a commercial library (NIST05). The relative proportions of individual components were expressed as percentage peak areas. Three replicates of each sample were analyzed.

2.7. Statistical Analyses

Microbiological, physicochemical, and quality data were subjected to one-way analysis of variance (ANOVA) after checking the normality distribution of the data with the Shapiro–Wilk test. Pairwise comparisons were performed by Tukey's test. Statistical significance was attributed to $p \leq 0.001$. Heat map analysis was used to graphically represent the distribution of VOCs in the different experimental breads. Through a hierarchical dendrogram and a representative map of the individual values of the relative peak areas in the data matrix represented in different colors, the differences between the various experimental trials were assessed. Each compound was represented by an intensity scale ranging from −1 (yellow) to >1 (red). All analyses were performed with XLStat software ver. 2019.2.2. (Addinsoft, New York, NY, USA).

3. Results

3.1. Microbial Evolution

Table 2 presents the levels of various microbial populations measured during the 12 h fermentation period. At the time of inoculation, the TMM population ranged from 6.55 to 7.16 Log CFU/g. The experimental dough B2 displayed the highest values at each sampling point (0, 3, 6, 9, and 12 h). An increasing trend was observed across all treatments, except for the B1 trial, which showed a slight decrease (about half a logarithmic cycle) between 9 and 12 h of fermentation. At the end of fermentation (12 h), three trials, C1, B2, and B3 had TMM values exceeding 8.5 Log CFU/g, while C2 and B1 had TMM values of 7.71 Log CFU/g and 8.02 Log CFU/g, respectively.

Table 2. Monitoring of microbial populations (expressed as Log CFU/g) of the experimental doughs (C1, C2, B1, B2, and B3) along the fermentation process (12 h).

Media (Microbial Groups)	Time (Hours)	Experimental Doughs					Statistical Significance
		C1	C2	B1	B2	B3	
PCA (Total mesophilic microorganisms)	0	6.55 ± 0.15 b	6.82 ± 0.16 ab	6.85 ± 0.21 ab	7.16 ± 0.22 a	6.80 ± 0.15 ab	*
	3	6.71 ± 0.12 b	7.06 ± 0.18 b	8.12 ± 0.16 a	8.29 ± 0.27 a	7.98 ± 0.17 a	***
	6	7.89 ± 0.24 b	7.09 ± 0.11 c	8.17 ± 0.08 ab	8.42 ± 0.24 a	8.21 ± 0.19 ab	***
	9	8.35 ± 0.21 a	7.48 ± 0.20 b	8.56 ± 0.06 a	8.47 ± 0.18 a	8.53 ± 0.09 c	***
	12	8.65 ± 0.17 a	7.71 ± 0.09 b	8.02 ± 0.14 b	8.70 ± 0.19 a	8.75 ± 0.18 a	***
mMRS (LAB rods)	0	6.43 ± 0.05 b	<1 d	6.97 ± 0.16 a	2.12 ± 0.09 c	7.04 ± 0.13 a	***
	3	6.97 ± 0.11 b	<1 d	8.98 ± 0.27 a	3.00 ± 0.19 c	8.52 ± 0.24 a	***
	6	8.01 ± 0.28 b	<1 d	8.50 ± 0.21 ab	4.50 ± 0.14 c	8.84 ± 0.26 a	***
	9	8.52 ± 0.23 a	<1 d	8.81 ± 0.26 a	4.85 ± 0.18 c	8.85 ± 0.12 b	***
	12	9.00 ± 0.20 a	4.00 ± 0.12 d	8.43 ± 0.16 b	5.50 ± 0.12 c	8.98 ± 0.13 a	***
M17 (LAB cocci)	0	6.67 ± 0.07 b	<1 c	7.11 ± 0.17 a	<1 c	7.07 ± 0.14 a	***
	3	7.35 ± 0.09 b	2.15 ± 0.06 d	8.80 ± 0.14 a	3.30 ± 013 c	8.53 ± 0.21 a	***
	6	8.28 ± 0.18 b	2.70 ± 0.17 d	8.63 ± 0.07 ab	4.51 ± 0.14 c	8.84 ± 0.09 a	***
	9	8.79 ± 0.15 a	4.00 ± 0.11 d	9.09 ± 0.04 a	5.02 ± 0.15 c	9.05 ± 0.11 b	***
	12	8.88 ± 0.20 a	4.18 ± 0.20 d	8.22 ± 0.16 b	5.44 ± 0.11 c	9.08 ± 0.10 a	***
SDB (Sourdough LAB)	0	6.05 ± 0.08 b	<2 c	6.88 ± 0.19 a	<2 c	6.70 ± 0.16 a	***
	3	6.87 ± 0.10 c	2.70 ± 0.16 d	8.98 ± 0.13 a	2.21 ± 0.18 e	7.73 ± 0.16 b	***
	6	8.70 ± 0.23 b	3.40 ± 0.11 c	9.78 ± 0.16 a	3.20 ± 0.17 c	8.42 ± 0.18 b	***
	9	8.65 ± 0.13 b	4.18 ± 0.20 d	9.25 ± 0.18 a	4.56 ± 0.15 d	8.60 ± 0.17 c	***
	12	8.80 ± 0.17 a	4.18 ± 0.16 c	8.91 ± 0.17 a	5.89 ± 0.14 b	8.62 ± 0.08 a	***

Table 2. *Cont.*

Media (Microbial Groups)	Time (Hours)	Experimental Doughs					Statistical Significance
		C1	C2	B1	B2	B3	
WL (Presumptive *Saccharomyces*)	0	2.20 ± 0.18 c	7.09 ± 0.19 a	2.30 ± 0.29 c	7.03 ± 0.13 a	6.30 ± 0.13 b	***
	3	2.35 ± 0.22 c	8.15 ± 0.08 a	2.40 ± 0.23 c	8.12 ± 0.16 a	7.25 ± 0.27 b	***
	6	2.70 ± 0.26 c	8.50 ± 0.14 a	2.55 ± 0.21 c	8.40 ± 0.15 a	7.42 ± 0.26 b	***
	9	3.10 ± 0.16 c	9.30 ± 0.09 a	3.15 ± 0.07 c	9.11 ± 0.11 a	7.65 ± 0.21 b	***
	12	3.50 ± 0.27 c	8.90 ± 0.16 a	3.45 ± 0.22 c	9.30 ± 0.10 a	8.11 ± 0.09 b	***
WL (Presumptive non-*Saccharomyces*)	0	2.70 ± 0.20 c	<2 d	6.66 ± 0.19 b	7.18 ± 0.12 a	6.55 ± 0.19 b	***
	3	<2 d	5.70 ± 0.26 c	7.77 ± 0.14 a	7.47 ± 0.11 ab	7.12 ± 0.18 b	***
	6	<2 c	6.74 ± 0.27 b	7.54 ± 0.16 a	6.70 ± 0.19 b	6.65 ± 0.21 b	***
	9	<2 c	6.21 ± 0.14 b	7.38 ± 0.24 a	6.17 ± 0.22 b	6.60 ± 0.10 b	***
	12	<2 d	5.74 ± 0.16 c	7.11 ± 0.17 a	5.70 ± 0.27 c	6.30 ± 0.07 b	***

Results indicate mean ± S.D. (standard deviation) of two independent experiments carried out 2 weeks apart. Abbreviations: PCA, plate count agar; mMRS, modified de Man, Rogosa, and Sharpe; SDB, sourdough bacteria; WL, Wallerstein Laboratory agar; C1, control trial inoculated with *Leuconostoc mesenteroides*; C2, control trial inoculated with *Saccharomyces cerevisiae*; B1, trial co-inoculated with *L. mesenteroides* and *Papiliotrema terrestris*; B2, trial co-inoculated with *S. cerevisiae* and *P. terrestris*; B3, trial co-inoculated with *L. mesenteroides*, *P. terrestris*, and *S. cerevisiae*. Data within a column followed by different letters are significantly different according to Tukey's test (*p*-value: ***, $p < 0.001$; *, $p < 0.05$).

Regarding the treatments inoculated with the starter strain of *L. mesenteroides* (C1, B1, and B3), initial microbial levels on mMRS ranged from 6.43 Log CFU/g (C1) to 7.04 Log CFU/g (B3), and on M17, they ranged from 6.67 (C1) to 7.11 Log CFU/g (B1). In the two trials without LAB inoculation (C2 and B2), only B2 recorded LAB rods slightly above the detection limit (2.12 Log CFU/g). In the LAB-inoculated trials (C1, B1, and B3), LAB rods showed an increasing trend, except for B1, which displayed slight fluctuations. An increasing trend in LAB levels was also observed in the B2 trial, despite the absence of LAB inoculation. This was attributed to the presence of indigenous LAB in the flour, which did not reach count values (<3 Log cycles) comparable to those in C1, B1, and B3. Only after 12 h did the LAB rods in the C2 treatment exceed the detection limits (4.00 Log CFU/g). LAB cocci showed a similar trend to the LAB rods. Microbial levels detected on M17 plates were comparable to those found by mMRS. However, in the C2 trial, LAB cocci were detected after 3 h but the levels observed in this trial and in B2 were about 3 Log cycles lower than those recorded in the LAB-inoculated trials. In B1, LAB cocci showed the same fluctuations, likely due to the interaction between *L. mesenteroides* and *P. terrestris*. Sourdough LAB populations exhibited an increasing trend in all trials, except for B1, which showed a slight decrease (−0.87 Log CFU/g) after 9 h of fermentation until the end of the process. As observed in mMRS and M17, after 6 h of fermentation, LAB populations exceeded 8 Log cycles. In all of the trials not inoculated with *L. mesenteroides*, LAB registered values slightly above 4 Log CFU/g.

The presumptive *Saccharomyces* populations showed an increasing trend in the inoculated trials (B2 and B3) until the end of fermentation. In contrast, the C2 control trial showed a slight decrease, with values dropping from 9.30 Log CFU/g to 8.90 Log CFU/g between 9 and 12 h of fermentation. In the doughs without *S. cerevisiae* inoculum (C1 and B1), the presumptive *Saccharomyces* counts were approximately 5 Log cycles lower on average. Nevertheless, an increasing trend was observed in both C1 and B1 treatments. Some variability was observed for the non-*Saccharomyces* yeast detection on WL agar. In treatments involving *P. terrestris* addition, values ranged from 6.55 Log CFU/g (B3) to 7.18 Log CFU/g (B2) immediately after inoculation. At the three-hour mark, there was an increase of about 1.11 Log cycles in B1, 0.29 in B2, and 0.57 in B3; however, from the sixth hour until the end of fermentation, recorded values decreased for all treatments inoculated with *P. terrestris,* reaching 7.11 Log CFU/g in B1, 5.70 Log CFU/g in B2, and 6.30 Log CFU/g in B3 after twelve hours of fermentation. The presumptive non-*Saccharomyces* were initially found at very low levels in C1 (2.70 Log CFU/g) and C2 (<2.00 Log CFU/g) treatments, which did not include *P. terrestris* inoculation. Non-*Saccharomyces* yeasts were detected only in the C2 trial from the third hour of fermentation onward. The highest value in the C2 control treatment was recorded at the 6-h detection point (6.74 Log CFU/g) but it decreased in subsequent measurements, reaching 5.74 Log CFU/g at the end of fermentation.

3.2. Monitoring of the Acidification Process

TTA and pH values recorded throughout the whole process are graphically represented in Figure 2.

Immediately after dough preparation, the average pH was 6.04 ± 0.01. After 3 h of fermentation, there was a pH drop ranging from 0.36 (C2) to 1.44 (B3) across all trials. After 6 h, the lowest pH value (4.33) was observed in the B3 trial, while the trial inoculated with *L. mesenteroides* (C1) had a slightly higher pH of 4.67. On the other hand, C2 and B2 trials showed pH values above 5.0. This trend continued after 9 h of fermentation, with pH values decreasing further for B1 and C1 by 0.3 and 0.37, respectively. After 12 h of fermentation, the pH values for C1 (4.24), B1 (4.35), and B3 (4.21) were similar and lower than those for the C2 and B2 trials (5.39 and 5.19, respectively). TTA values showed an inverse trend compared to the pH values. TTA values tended to increase as pH decreased, requiring more 0.1 N NaOH to neutralize and achieve the characteristic color change for the analysis [24]. At the end of the fermentation process, the highest TTA value was recorded

in the B3 trial (10.48 mL of NaOH 0.1 N), while the C2 trial had the lowest value (5.12 mL of NaOH 0.1 N).

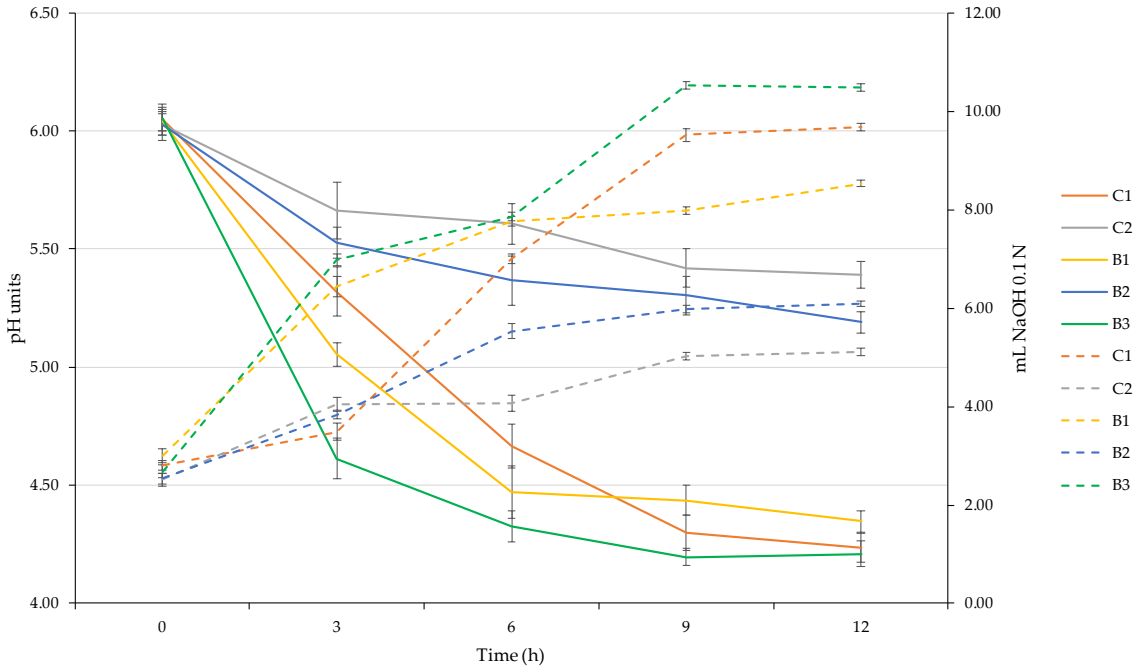

Figure 2. Monitoring of pH and TTA during the fermentation process. Abbreviations: C1, control trial inoculated with *Leuconostoc mesenteroides*; C2, control trial inoculated with *Saccharomyces cerevisiae*; B1, trial co-inoculated with *L. mesenteroides* and *Papiliotrema terrestris*; B2, trial co-inoculated with *S. cerevisiae* and *P. terrestris*; B3, trial co-inoculated with *L. mesenteroides*, *P. terrestris*, and *S. cerevisiae*.

3.3. Qualitative Analyses of Doughs

The results regarding weight loss due to CO_2 emission are shown in Figure 3. After 3 h of fermentation, the highest weight loss was recorded for the B2 trial (0.18 g of CO_2), whereas no loss occurred in the B1 trial. After 6 h of fermentation, the greatest weight loss was again observed in the B2 trial (0.44 g of CO_2), while C1 (0.05 g of CO_2) and B1 (0.01 g of CO_2) showed the lowest weight loss. The B2 treatment continued to record the highest weight loss after 9 h (0.57 g of CO_2) but these values were comparable to those observed in C2 (0.53 g of CO_2), which were similar to those in the B3 trial (0.45 g of CO_2). Consistent with the previous time points, weight loss in C1 and B1 was lower, at 0.14 and 0.06 g of CO_2, respectively.

By the end of the 12-h fermentation, the trials C2, B2, and B3 had similar weight loss values, ranging from 0.61 to 0.66 g of CO_2. In contrast, C1 (0.16 g of CO_2) and B1 (0.07 g of CO_2) exhibited the lowest values, consistent with previous observations. In general, treatments inoculated with *S. cerevisiae* showed the greatest weight loss. Notably, after 9 h, the B2 trial (co-inoculated with *S. cerevisiae* and *P. terrestris*) recorded the highest value, comparable to the control trial (C2) inoculated with *S. cerevisiae* alone. A similar trend was observed in the increase in dough volume during fermentation (Figure 4). The trials inoculated with *L. mesenteroides* (C1 and B1) showed lower volume increases compared to those inoculated with *S. cerevisiae* (C2, B2, and B3); however, at 6 h, the trial with mixed inoculation of *S. cerevisiae*, *L. mesenteroides*, and *P. terrestris* (B3) showed the greatest volume increase (142.83 mL). After 9 and 12 h, the data for the B3 trial aligned with the C2 control trial inoculated with *S. cerevisiae* alone. The volume increase mirrored the trend observed for weight loss in the various doughs at different detection points.

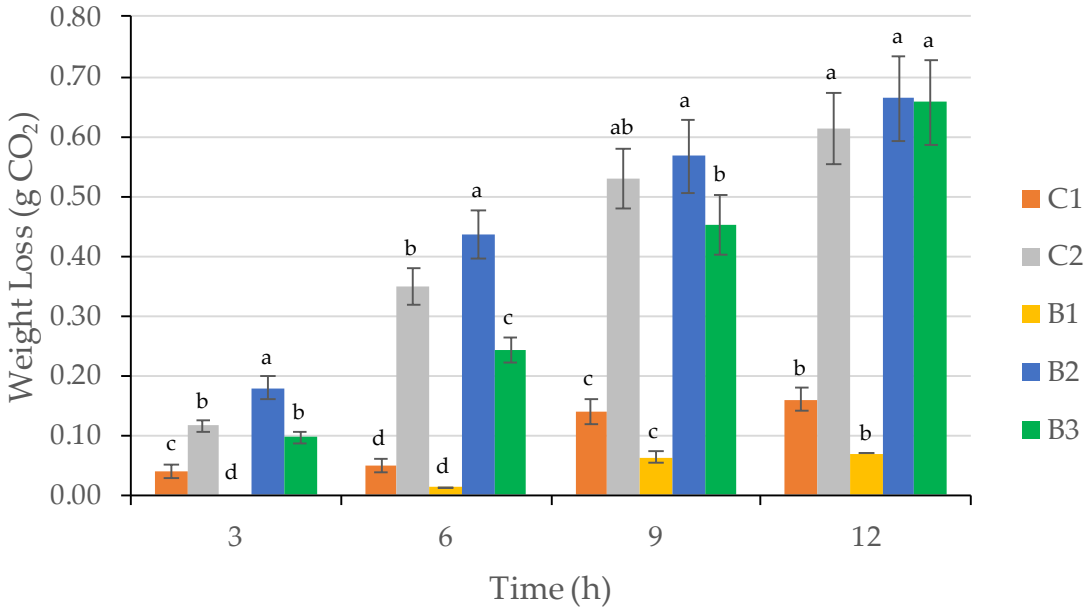

Figure 3. Dough weight loss (g CO_2) throughout the fermentation process. Abbreviations: C1, control trial inoculated with *Leuconostoc mesenteroides*; C2, control trial inoculated with *Saccharomyces cerevisiae*; B1, trial co-inoculated with *L. mesenteroides* and *Papiliotrema terrestris*; B2, trial co-inoculated with *S. cerevisiae* and *P. terrestris*; B3, trial co-inoculated with *L. mesenteroides*, *P. terrestris*, and *S. cerevisiae*. Different letters for each fermentation time between trials indicate statistically significant differences according to Tukey's test for $p \leq 0.001$.

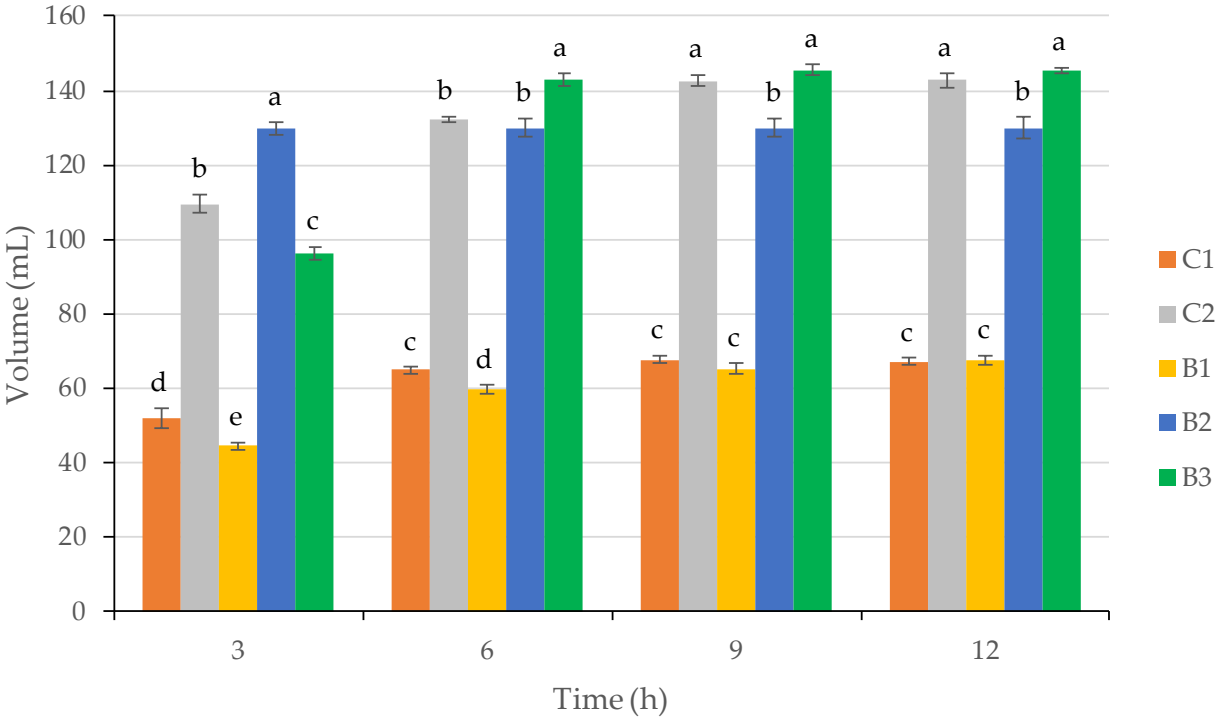

Figure 4. Dough volume (mL) detected throughout the fermentation process. Abbreviations: C1, control trial inoculated with *Leuconostoc mesenteroides*; C2, control trial inoculated with *Saccharomyces cerevisiae*; B1, trial co-inoculated with *L. mesenteroides* and *Papiliotrema terrestris*; B2, trial co-inoculated with *S. cerevisiae* and *P. terrestris*; B3, trial co-inoculated with *L. mesenteroides*, *P. terrestris*, and *S. cerevisiae*. Different letters for each fermentation time between trials indicate statistically significant differences according to Tukey's test for $p \leq 0.001$.

3.4. Qualitative Analyses of Breads After Baking

Bread weight loss after baking, which ranged from 14.46% to 18.12%, did not show any significant differences between the samples (Table 3). However, the color analysis revealed significant differences between treatments at both the crust and crumb levels, regarding the parameters L* (brightness), a* (red–green variation), and b* (yellow–blue variation).

For C1 baked bread, the L* parameter of the crust showed the highest values (75.91) compared to all other trials. As a consequence, ΔL* was the lowest (17.63) for this trial, as the crust color of this experimental bread was the closest to the white standard. Conversely, the a* parameter for C1 bread showed the lowest values (1.75), while the other trials had values ranging from 5.44 to 6.97, indicating a greater tendency toward red. For the b* parameter, the highest value was recorded for the B3 treatment (30.05), while the lowest was for C1 (19.22).

The crumb color parameters showed slightly different trends compared to the crust. Regarding the L* parameter, the breads from trials C1, B1, and B3 showed the highest values, ranging from 65.44 to 69.52. The lowest values were recorded for the B2 bread (51.68), while the C2 trial showed intermediate L* values (58.75); these two experimental breads showed the highest ΔL* value (41.86 and 34.79, for B2 and C2, respectively) as they had the darkest crumbs compared to the other trials. Regarding the a* parameter, all treatments showed negative values, indicating that the crumb color tended slightly toward green. Specifically, B2 showed the highest values (−1.90), with intermediate values recorded for C2, B1, and B3 breads (−2.40–−2.53), and the lowest for C1 (−2.95). The crumbs of all breads tended toward yellow, as indicated by the b* parameter, with variable results between treatments. The highest values were recorded for C1, B2, and B3 trials (18.14 to 18.37), and the lowest for the B2 bread (16.21).

With regard to specific volume, the highest values were recorded for trials C2 and B3, with 3.57 cm^3/g and 3.51 cm^3/g, respectively, followed by B2, which had a specific volume value (3.30 cm^3/g) in an intermediate statistical position between the two breads mentioned above and the others. Finally, B1, the experiment in which *S. cerevisiae* was not inoculated, recorded the lowest value at 2.82 cm^3/g. The image analysis conducted on the central slices of each loaf of bread after baking and cooling revealed significant differences between the trials in terms of cell density and mean cell area, although the void fraction did not show statistically significant differences. The evaluation of cell density and mean cell area distinctly separated the C1 and B2 breads. Specifically, the C1 trial exhibited 70.52 cells/cm^2, whereas B2 had a reduced value of 41.63 cells/cm^2. Conversely, the mean cell area was lower in C1 (0.59 mm^2) compared to B2 (1.08 cm^2). This phenomenon is typical for doughs fermented with LAB starter cultures. In contrast, cell density decreased and the mean cell area increased when *S. cerevisiae* was used as the leavening agent. The remaining trials showed intermediate values.

Table 3. Bread weight loss, specific volume, color, and image analysis.

Parameters		Experimental Breads					Statistical Significance
		C1	**C2**	**B1**	**B2**	**B3**	
Weight loss (%)		16.81 ± 1.21 a	17.10 ± 1.08 a	16.02 ± 1.40 a	18.12 ± 2.26 a	14.46 ± 1.30 a	n.s.
Crust color							
	Lightness (L*)	75.91± 2.82 a	55.92 ± 1.49 b	59.07 ± 5.27 b	61.72 ± 2.11 b	59.34 ± 0.33 b	***
	Red–green (a*)	1.75 ± 0.72 b	5.76 ± 0.54 a	5.44 ± 1.00 a	5.68 ± 0.92 a	6.97 ± 0.68 a	***
	Yellow–blue (b*)	19.22 ± 1.38 c	25.21 ±0.20 b	27.36 ± 1.13 ab	26.21 ± 1.45 b	30.05 ± 1.22 a	***
	ΔL*	17.63	37.62	34.47	31.82	34.20	
Crumb color							
	Lightness (L*)	65.44 ± 4.04 a	58.75 ± 1.19 b	69.52 ± 2.23 a	51.68 ± 2.10 c	65.78 ± 1.59 a	***
	Red–green (a*)	−2.95 ± 0.08 c	−2.48 ± 0.03 b	−2.53 ± 0.14 b	−1.90 ± 0.20 a	−2.40 ± 0.09 b	***
	Yellow–blue (b*)	18.37 ± 0.18 a	17.29 ± 0.58 ab	18.34 ± 0.20 a	16.21 ± 0.75 b	18.14 ± 0.49 a	***
	ΔL*	28.10	34.79	24.02	41.86	27.76	
	Specific volume (cm^3/g)	3.03 ± 0.22 bc	3.57 ± 0.08 a	2.82 ± 0.21 c	3.30 ± 0.03 ab	3.51 ± 0.17 a	***
Image analysis							
	Void fraction (%)	39.42 ± 6.43 a	42.41 ± 2.28 a	43.80 ± 3.23 a	44.64 ± 0.49 a	39.38 ± 0.93 a	n.s.
	Cell density (n/cm^2)	70.52 ± 14.36 a	49.33 ± 7.34 ab	46.48 ± 6.79 ab	41.63 ± 3.98 b	57.48 ± 11.16 ab	*
	Mean cell area (mm^2)	0.59 ± 0.24 b	0.88 ± 0.18 ab	0.96 ± 0.11 ab	1.08 ± 0.10 a	0.70 ± 0.14 ab	*

Results indicate mean values ± S.D. (standard deviation) of six determinations (carried out in three technical repeats for two independent experiments). Data within a row followed by different letters are significantly different according to Tukey's test (*p*-value: ***, $p < 0.001$; *, $p < 0.05$). Abbreviations: C1, control trial inoculated with *Leuconostoc mesenteroides*; C2, control trial inoculated with *Saccharomyces cerevisiae*; B1, trial co-inoculated with *L. mesenteroides* and *Papiliotrema terrestris*; B2, trial co-inoculated with *S. cerevisiae* and *P. terrestris*; B3, trial co-inoculated with *L. mesenteroides*, *P. terrestris*, and *S. cerevisiae;* n.s., not significant.

3.5. Volatile Organic Compounds (VOCs)

The results concerning VOC composition are graphically presented in Figure 5. The heat map grouped the five trials into three clusters based on the VOC profile detected by GC/MS after SPME.

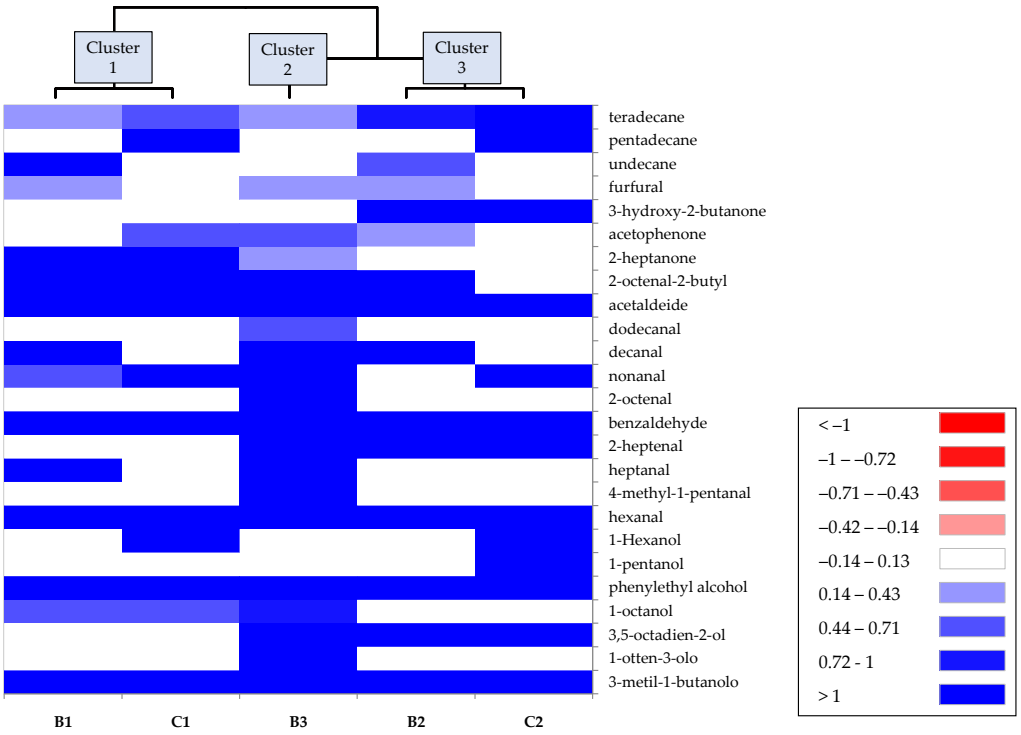

Figure 5. Volatile organic compound distribution between different experimental breads after baking. Abbreviations: C1, control trial inoculated with *Leuconostoc mesenteroides*; C2, control trial inoculated with *Saccharomyces cerevisiae*; B1, trial co-inoculated with *L. mesenteroides* and *Papiliotrema terrestris*; B2, trial co-inoculated with *S. cerevisiae* and *P. terrestris*; B3, trial co-inoculated with *L. mesenteroides*, *P. terrestris*, and *S. cerevisiae*.

Specifically, cluster 1 was represented by B1 and C1 trials, cluster 2 by the B3 treatment, and cluster 3 by B2 and C2 trials. Cluster 1 was characterized by higher amounts of hexanal and nonanal; cluster 2 by benzaldehyde and 3-hydroxy-2-butanone; and cluster 3 by higher amounts of 3-methyl-1-butanol. Notably, in the experimental breads inoculated with *P. terrestris* (B1 and B2), the VOC profile did not show any significant differences compared to the respective controls (C1 and C2). A distinct aroma profile was observed for B3, which involved the mixed inoculum of all three microbial strains (*S. cerevisae*, *L. mesenteroides*, and *P. terrestris*).

4. Discussion

Over the past decade, one of the major challenges has been researching and applying potential non-*Saccharomyces* yeast starters and co-starters to improve the quality, aroma, and sensory characteristics of bakery products [7,8,25].

This study evaluated the suitability of the *P. terrestris* strain PT22AV for breadmaking in collaboration with Agroventures Srl. *Papiliotrema terrestris* PT22AV was used in co-culture with a commercial strain of *S. cerevisiae* and a commercial formulation of the LAB starter *L. mesenteroides*. The potential of the strain in breadmaking is related to its ability to produce exopolysaccharides, as described by Santra and Banerjee [12]. Microbial-derived exopolysaccharides have numerous effects on bakery products. By binding with various chemical components in the dough, they can improve bread's structure, volume, and crumb softness, which reduces staling, and thus improves the shelf life of the product [26]. This

research focused on evaluating the interaction between *P. terrestris* PT22AV and two commercial leavening agents, *S. cerevisiae* and *L. mesenteroides*, under co-inoculation conditions.

The monitored microbial populations revealed differences based on the inoculation method used for each trial. In the experimental doughs inoculated with *P. terrestris* PT22AV (B1, B2, and B3), yeast growth was observed only during the first 3 h of fermentation. A similar observation was reported by Alfonzo et al. [19] in doughs co-inoculated with *S. cerevisiae* and *P. terrestris*, where the non-conventional yeast strain exhibited an increase in microbial cell density by approximately half a Log cycle after 2 h of fermentation. However, at subsequent detection points, the PT22AV strain showed a decrease in levels, likely due to competition with the other starter strains. The decline in non-*Saccharomyces* yeasts during dough fermentation is a common occurrence in co-inoculation with *S. cerevisiae*. Li et al. [27] observed a significant reduction in *T. delbrueckii* (non-*Saccharomyces*) yeast during co-culture fermentation with *S. cerevisiae*, although the non-*Saccharomyces* yeast remained viable. This phenomenon can be attributed to factors such as peptide production by *S. cerevisiae* [28] or spatial competition [29]. In our study, when *P. terrestris* was co-inoculated with *L. mesenteroides* (B1), the growth dynamics of the non-*Saccharomyces* yeast showed a similar pattern to those in trials inoculated with *S. cerevisiae* (B2 and B3), although microbial densities of the presumptive non-*Saccharomyces* in B1 were higher than in the B2 and B3 trials. The early presence of *L. mesenteroides* at high densities likely created a more favorable environment for *P. terrestris* [30].

The acidification dynamics of the doughs, measured by pH and TTA, were found to be influenced by the type of co-inoculum used. The presence of *L. mesenteroides* in the C1, B1, and B3 trials resulted in a greater degree of dough acidification compared to the trials inoculated with yeasts (C2 and B2). The rapid acidification capacity of LAB is closely linked to the specific strain inoculated; however, this documented phenomenon creates a selective environment that can influence the final bread's characteristics [31]. The B3 trial, inoculated with a combination of *P. terrestris*, *S. cerevisiae*, and *L. mesenteroides*, exhibited the highest degree of acidification. The interaction levels between *Saccharomyces*, non-*Saccharomyces*, and LAB are complex. Still, it is likely that the high initial levels of the three species in the dough led to a series of positive interactions [32], resulting in rapid dough acidification. In addition, this effect was more pronounced when *L. mesenteroides* was inoculated individually (C1).

During the fermentation process, the B2 trial (*S. cerevisiae* + *P. terrestris*) exhibited the highest CO_2 production compared to the C2 control (*S. cerevisiae*), aligning with the findings of Alfonzo et al. [19]. The treatments inoculated with *L. mesenteroides* (C1 and B1) exhibited reduced CO_2 production, attributed to the lower metabolic activity of the LAB starter strain compared to the CO_2 production observed in the *S. cerevisiae* trial. This phenomenon is expected, as yeasts typically produce more CO_2 than LAB [33]. However, extending the fermentation time to 12 h resulted in comparable CO_2 emissions in doughs inoculated with *S. cerevisiae* (C2, B2, and B3), both in doughs with only *P. terrestris* (B2) and in mixed inoculation conditions (*S. cerevisiae* + *L. mesenteroides* + *P. terrestris*) in the B3 treatment. Xu et al. [34] demonstrated that *S. cerevisiae* positively impacts CO_2 production, whether in sourdough starter, co-inoculated with lactobacilli, or as a single inoculum. Non-*Saccharomyces* yeasts generally have lower CO_2 production capacity, minimally affecting the overall fermentation process. There is, however, a discrepancy regarding the interaction between brewer's yeast and LAB. Brandt et al. [35] reported that *S. cerevisiae* and heterofermentative LAB equally contribute to CO_2 production, while other authors, including Gobbetti et al. [36,37] and Gänzle et al. [38], have suggested that yeasts produce more CO_2 than LAB. This discrepancy explains why some bakers add *S. cerevisiae* to sourdough to address leavening issues [34]. Indeed, the highest volume increase was observed in doughs inoculated with *S. cerevisiae* (C2 and B2), and *P. terrestris* did not influence this parameter; however, after 9 h, the volume increase was lower in the B2 trial co-inoculated with *S. cerevisiae* and *P. terrestris*. This is consistent with Li et al. [27], who reported a slight volume reduction in dough inoculated with *S. cerevisiae* and *T. delbruekii* in a 1:1 ratio.

The color of the breads' crust and crumb after baking was influenced by the type of co-inoculum, with significant differences observed in the L*, a*, and b* coordinates used to define the CIELAB space. Despite these differences, the effect of *P. terrestris* on the crust and crumb color was not significant. The variations were attributed to the starter strain used. Both *L. mesenteroides* and *S. cerevisiae* shifted the crust and crumb color toward white but with a lower brightness level based on the L* coordinate values. This trend is also consistent with the findings of Makambai et al. [39].

Specific volume analyses revealed that the trials in which *S. cerevisiae* was inoculated exhibited the highest values (C2, B2, and B3). Although the B2 trial also included *P. terrestris,* and in the B3 trial there were both *P. terrestris* and *L. mesenteroides*, the C1 (*L. mesenteroides* alone) and B1 (*L. mesenteroides* co-inoculated with *P. terrestris*) trials demonstrated that the increased specific volume was mainly due to *S. cerevisiae.* These findings are in line with those reported by Condessa et al. [8], where a trial fermentation conducted with a commercial *S. cerevisiae* strain showed a higher specific volume than that fermented by non-*Saccharomyces* strains.

Analyzing the crumb image of different experimental breads is a key parameter in determining product quality after baking [40]. Several authors have indicated that void fraction, cell density, and mean cell area are primarily influenced by factors related to the type of flour and the dough production processes [41]. In the conducted breadmaking tests, the only variation was the combinations of the inocula with different microorganisms. Surprisingly, no differences were observed in the void fraction, indicating that the interactions between the different strains inoculated in the doughs did not result in significant differences in this parameter compared to the control bread.

Numerous studies have shown that microbial cooperation in mixed inocula is frequent. For instance, Yu et al. [42] investigated the effect of combining LAB (*L. rossiae*, *L. brevis*, and *L. plantarum*) with *S. cerevisiae* on various parameters characterizing the structure of bread crumbs. They observed a significant increase in the void fractions in breads made from mixed inocula dough, indicating that LAB and yeast strain cooperation can be beneficial. The cell density aspect showed that bread made from a single inoculum of *L. mesenteroides* had a higher number of cells per cm^2 of the slice, consistent with findings previously reported [43]; however, cell density decreased when *S. cerevisiae* was inoculated into the dough. A unique phenomenon was observed when *L. mesenteroides* was co-inoculated with *P. terrestris*, resulting in a cell density similar to that of breads fermented individually with *S. cerevisiae* or in combination with *L. mesenteroides*. This was further confirmed by the mean cell area, where significant differences were observed between breads made with *S. cerevisiae* alone and those co-inoculated with *P. terrestris*. These observed differences are attributed to the fermentation agent used, as commercial yeasts often exhibit a better fermentation ability, leading to a higher CO_2 production rate. This exerts considerable stress on the cell wall, causing expansion and subsequent disruption [44]. This phenomenon results in the formation of large bubbles, reducing the cell density and increasing the average area size [42]. A higher cell density and smaller area size are related to the fermentation performance of *L. mesenteroides*, which is not comparable to that of commercial yeast. The fermentation process is slower with sourdough, resulting in a lower CO_2 production rate [42,45].

The aroma profiles of bread result from a combination of numerous volatile compounds derived from raw materials, such as flour, which are produced or modified during fermentation and baking. Various compounds from different chemical classes, including alcohols, aldehydes, ketones, furans, and hydrocarbons, were identified in all experimental breads. The presence of these compounds has been documented in multiple studies by several authors: Gaglio et al. [46]; Giannone et al. [47]; Paterson [48]; Pico et al. [49]. Among the different production methods, breads with the most complex aroma profiles were those made with doughs inoculated with *L. mesenteroides*, *S. cerevisiae*, and *P. terrestris* (B3). In the B3 trial, 21 compounds were identified, while 13 to 15 compounds were found in the other breads. This complexity is likely due to the use of mixed cultures in B3 production. The

cooperation of LAB and yeast strains has been shown to enhance the composition of volatile compounds, creating a richer aroma profile [37,50,51]. Meignen et al. [51] demonstrated that mixed cultures offer several advantages, particularly in terms of flavor and consistency over time, compared to bread made with commercial *S. cerevisiae* strains. However, regardless of the production method, no significant differences in flavor profiles were observed when *P. terrestris* was included in the dough.

In general, the aroma profiles were similar, likely influenced by microbial composition as well as interactions between the baking process and ingredients [52]. These interactions are crucial for maintaining process stability and producing a variety of regional specialties that meet consumer and commercial demands [53]. For example, ingredient selection in sourdough bread significantly impacts the production of VOCs. Heinio et al. [54] studied the differences in VOC profiles between bread fermented with sourdough and rye flours, showing notable variations despite similar production processes. Additionally, the types of cereal flours, both endogenous and exogenous cereal components, and the processing steps like heat treatment during baking significantly influence the generation of flavor compounds in bread [48].

5. Conclusions

This study confirmed that even with the same raw materials, using different leavening agents can produce breads with varied characteristics. Specifically, co-inoculating *S. cerevisiae* with *L. mesentorides* yielded a higher quality product. This was evident in the rapid acidification of the dough, the technological characteristics of the bread after baking, and its sensory attributes. Adding *L. mesenteroides* to the *S. cerevisiae* yeast strain resulted in a diversified and improved product, representing a technological innovation that should be considered in industrial breadmaking processes, which currently rely solely on *S. cerevisiae*. However, using *P. terrestris* PT22AV did not lead to any significant technological or aromatic differences in the final product. These findings suggest a potential limitation for the use of several non-*Saccharomyces* yeasts in bakery biotechnology. Further studies are needed to understand the nutritional needs of *P. terrestris* PT22AV, which will help enhance its adaptability to the dough environment and maximize its exopolysaccharide production. Future evaluations should focus on parameters such as increased dough viscosity, crumb softness, delayed starch retrogradation, and increased bread volume to justify the addition of *P. terrestris* PT22AV after addressing its nutritional requirements. Exploring new starter or co-starter cultures and validating protocols for using microorganisms in the breadmaking process is crucial to meeting demands and improving product quality. Lastly, the technological and aromatic characterization of products made from dough fermented with the PT22AV strain, co-inoculated with *S. cerevisiae* or *L. mesenteroides*, showed no significant changes in fermentation characteristics compared to those made with conventional microbial strains. This finding rules out the risk that contamination with viable cells of the *P. terrestris* strain PT22AV—derived from its use as a biocontrol agent on wheat (in-field stage)—could have altered the technological characteristics of the doughs during breadmaking.

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