

## Article

# Impact of Lactic Acid Bacteria on Sour India Pale Ale (IPA) Fermentation: Growth Dynamics, Acidification, and Flavor Modulation

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## Abstract

Sour beer production is strongly influenced by the choice of lactic acid bacteria (LAB), yet few studies have systematically compared strain-specific contributions under controlled kettle souring conditions. This study evaluated the fermentation performance and flavor-modulating potential of three LAB species—*Lactocaseibacillus paracasei*, *Pediococcus pentosaceus*, and *Leuconostoc mesenteroides*—in sour India Pale Ale (IPA) brewing. Growing assessments showed that *P. pentosaceus* exhibited the most rapid and stable proliferation, while *L. mesenteroides* required a longer adaptation period. Acidification trials demonstrated that *L. paracasei* achieved the lowest pH (3.26–3.43), contributing to intense sourness, whereas *P. pentosaceus* and *L. mesenteroides* yielded milder acidity (pH 3.41–3.65). Gas chromatography-mass spectrometry showed that *P. pentosaceus* and *L. mesenteroides* produced significantly higher levels of fruity and floral esters, including 2-pentanol propanoate, which was approximately 4-fold higher than in the control. Principal component analysis further distinguished the beers according to their volatile profiles. These findings highlight the strain-specific potential of LAB in sour beer brewing and provide practical guidance for flavor differentiation in craft beer production.

**Keywords:** acidification; craft beer production; fermentation; LAB; sour beer; volatile compounds



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## 1. Introduction

Beer is one of the most popular alcoholic beverages worldwide, enjoyed by people across diverse cultures. In recent decades, the craft beer movement has significantly transformed the industry, emphasizing small-scale production, artisanal techniques, and unique flavors [1–3]. Craft beer has seen explosive global growth, known for its wide diversity [4]. Among the styles gaining popularity are sour beers, recognized for their distinctive acidity and fruity notes [5].

Sour beers are generally categorized into two main styles: European sour ales and American wild ales. European sour ales, such as lambic, gueuze, and Berliner Weisse, are known for their low bitterness and balanced sourness, produced through spontaneous fermentation [5–7]. On the other hand, American wild ales are brewed by intentionally

introducing non-traditional microorganisms, often aged in oak barrels, resulting in a highly diverse range of flavors [8,9]. This modern style is highly diverse, with each beer displaying unique characteristics, often influenced by the inclusion of various yeasts and bacteria, such as *Saccharomyces* spp., *Brettanomyces* spp., *Lactobacillus* spp., and *Pediococcus* spp. [10,11].

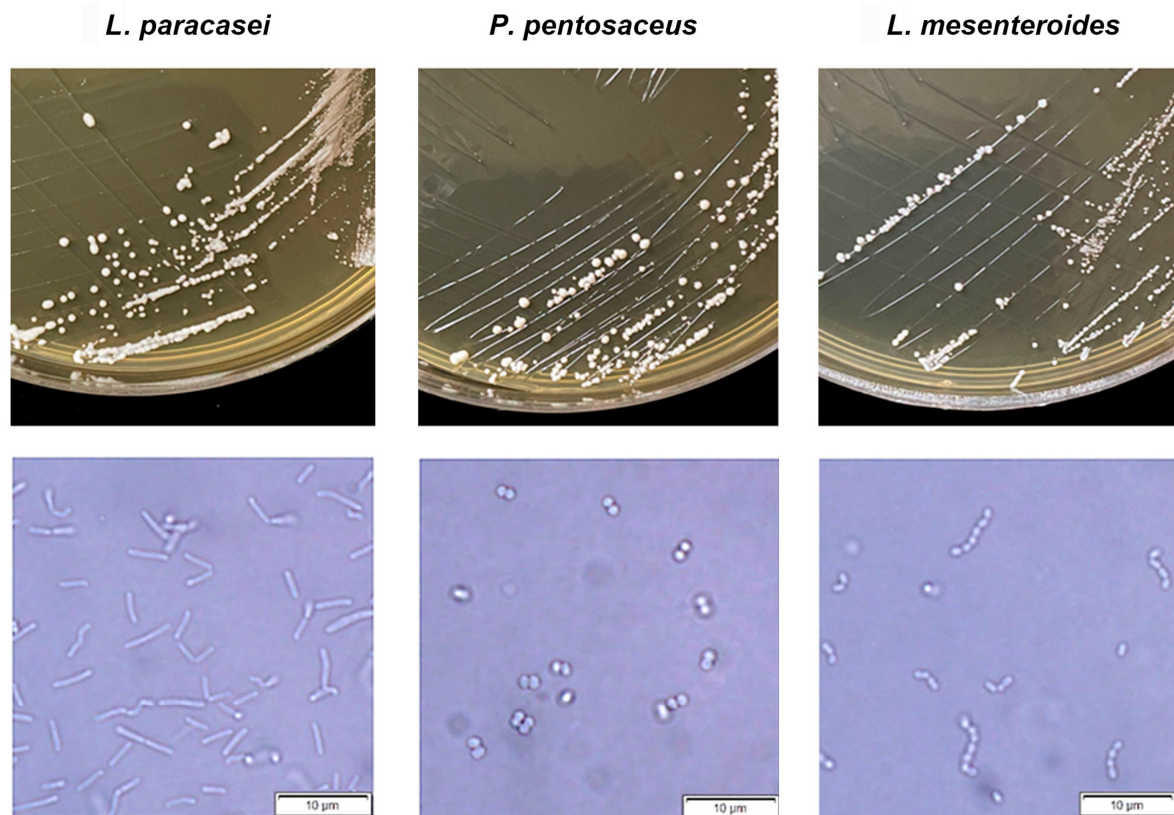
Modern techniques have been developed to streamline sour beer production beyond traditional wild fermentation [5]. “Kettle souring” where wort is cooled after mashing, inoculated with lactic acid bacteria (LAB), and fermented to the desired acidity before boiling [12]. This approach allows for faster production and better microbial control, with boiling halting fermentation and preventing further bacterial growth [9]. However, it can tie up equipment and requires acid-tolerant yeast [5]. “Mixed fermentation” uses multiple bacterial and yeast strains, often maturing in wooden barrels, enhancing complexity but requiring longer maturation [8,9,13]. Non-brewing yeasts like *Lachancea thermotolerans* and *Wickerhamomyces anomalus* can be used in primary fermentation to ferment and acidify simultaneously, reducing contamination risks [14].

Lactic acid bacteria (LAB) are Gram-positive microorganisms that primarily metabolize carbohydrates and exhibit a high tolerance to low pH [15]. With a long-standing role in food fermentation, LAB are widely used in the production and preservation of various products such as yogurt, cheese, and kimchi, where they enhance flavor, safety, and nutritional value [16]. Common genera include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Enterococcus*, and *Weissella* [17]. The functional contributions of different LAB species during fermentation are influenced by their varying capacities to utilize polysaccharides. While traditional classification was based on phenotypic traits, modern taxonomy increasingly relies on genetic approaches—particularly 16S rRNA sequencing—for more precise and reliable identification [18].

In the context of beer brewing, LAB contributes significantly to acidification, shaping the profiles of the sour beer style [19]. Among them, *Lactobacillus* and *Pediococcus* are most commonly used due to their reliable acid production and adaptability to brewing environments. In contrast, other genera such as *Leuconostoc*, *Enterococcus*, and *Weissella* are less frequently used, potentially due to lower acid production, undesirable flavor compounds, or safety concerns related to pathogenicity [20–22]. Overall, LAB are essential in food and beverage fermentation, offering benefits in preservation, flavor development, and nutritional enhancement.

In this study, our primary aim was to compare the fermentation performance and flavor contributions of three LAB species—*Lactobacillus paracasei*, *Pediococcus pentosaceus*, and *Leuconostoc mesenteroides* (Figure 1)—for sour beer production via kettle souring. These LAB species were selected based on their established role in sour beer brewing or wine fermentation [19,23], and all belong to the non-spore-forming *Lactobacillaceae* family. *L. paracasei* is rod-shaped, a facultative anaerobic that ferments sugars homofermentatively to produce lactic acid, contributing intense sourness [24,25]. *P. pentosaceus*, a spherical, homofermentative species, has been reported to support yeast growth and improve wort filtration during brewing [26–28]. *L. mesenteroides*, in contrast, is a heterofermentative coccus that produces lactic acid, CO<sub>2</sub>, and ethanol, and is known for its buttery aroma contribution via diacetyl. It is widely used in fermenting sauerkraut, kimchi, and dairy [29]. By examining these three species in parallel, this study provides comparative insights into their acidification kinetics, flavor compound profiles, and overall suitability for crafting distinct sour beer styles. *L. paracasei* exhibited the most rapid acidification and produced the sourest beer, while *P. pentosaceus* demonstrated the fastest and most stable growth and contributed the most complex and floral aroma profile, notably rich in phenylethyl alcohol and esters. *L. mesenteroides* grew more slowly and produced a milder beer with balanced acidity. These findings suggest that *P. pentosaceus* is well-suited for fruit-forward sour

beers, *L. paracasei* for intensely sour profiles, and *L. mesenteroides* for mild, well-balanced options. This work aligns with the growing industry interest in diversifying sour beer profiles and developing more precise fermentation strategies to meet consumer demand for novel, flavor-forward craft beverages.



**Figure 1.** Appearance and morphology of lactic acid bacteria colonies. The upper panel displays the appearance of lactic acid bacteria grown on MRS agar plates. From left to right: *L. paracasei*, *P. pentosaceus*, and *L. mesenteroides*. The bottom panel provides a magnified view of the bacteria's morphology observed under a 1600 $\times$  oil immersion lens.

This study aimed not only to evaluate fermentation performance and flavor contributions of different LAB strains, but also to clarify their acidification kinetics, growth stability, and strain-specific influence on volatile profiles. By doing so, we provide brewers with targeted insights for selecting suitable LAB species under controlled kettle souring conditions, which has not been systematically studied before.

## 2. Materials and Methods

### 2.1. Lactic Acid Bacteria Strains and Culture Conditions

The three lactic acid bacteria (LAB) strains used in the experiment—*L. paracasei*, *P. pentosaceus*, and *L. mesenteroides*—were gifted by Taiwan Ale Beer Co., Ltd. The strains were preserved in 15% glycerol at  $-80^{\circ}\text{C}$  and cultured in de Man–Rogosa–Sharpe (MRS) medium (BD Difco™ Lactobacilli MRS Broth, Thermo Fisher Scientific, Waltham, MA, USA), which is specifically designed for LAB. The MRS medium for *P. pentosaceus* and *L. mesenteroides* was adjusted to pH 5.5 and cultured at  $30^{\circ}\text{C}$ , while *L. paracasei* was cultured in MRS medium adjusted to pH 6.5 at  $37^{\circ}\text{C}$ .

## 2.2. 16S rRNA Gene Sequencing of LAB Species

The identity of the three LAB species was confirmed by amplifying their 16S rRNA gene sequences using bacterial universal primers, followed by sequencing and comparison with entries in the NCBI database. Colony PCR was performed using Taq DNA Polymerase 2× Master Mix RED (Ampliqon, Odense, Denmark) and universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CTACGGCTACCTTGTACGA-3'). The PCR conditions were: 95 °C for 15 min (initial denaturation), followed by 35 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 90 s, with a final extension at 72 °C for 10 min. The PCR products were run on a 1% agarose gel, stained with SYBR green, and visualized using a gel documentation system (Gel Logic 212 PRO, Bruker BioSpin, Billerica, MA, USA).

Remaining PCR products were purified using the FB PCR Clean Up/Gel Extraction Kit (FairBiotech, Taiwan), quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientific), then cloned, sequenced, and identified via comparison with NCBI database entries.

## 2.3. Growth Curve of LAB

LAB cultures were initially grown in 100 mL of MRS medium with an initial OD<sub>600</sub> of 0.00 (Biowave Cell Density Meter CO8000, Biochrom Ltd., Cambridge, UK). The suspension was then adjusted to an OD<sub>600</sub> of 0.10, and absorbance readings were taken every 2 h for 24 h, with a final reading at 48 h to monitor growth. OD<sub>600</sub> values were used to estimate total cell density, while viable cell counts were determined by plating serial dilutions on MRS agar. All experiments were performed in triplicate for each LAB species to ensure reproducibility and statistical reliability.

To prepare a saturated bacterial suspension, a single colony was first inoculated into 3 mL MRS broth and cultured overnight. The culture was then transferred to 20 mL of fresh MRS for further growth. Absorbance was measured at OD<sub>600</sub>, using un-inoculated MRS as a baseline (OD<sub>600</sub> = 0.00). Based on the OD<sub>600</sub> readings, the appropriate volume was calculated to inoculate 100 mL of MRS to achieve an initial OD<sub>600</sub> of 0.10. Samples were collected every 2 h to monitor growth, and a growth curve was generated from the resulting measurements.

## 2.4. Brewing of Sour IPA

The brewing process began with mashing, where 5 kg of crushed malt (80% pale ale, 20% wheat malt; Castle Malting, Mons, Belgium) was mixed with 25 L of filtered water at 43 °C in the 20L-brewing kettle (Speidel Braumeister, Ofterdingen, Germany). The temperature was gradually raised to 68 °C and held for 60 min, followed by a final step at 76 °C for 10 min. After mashing, the wort was sparged and briefly boiled (3–5 min) to reduce microbial contamination, then cooled to ~30 °C for kettle souring.

For souring, the cooled wort was transferred to sterilized barrels and inoculated with saturated LAB cultures (~10<sup>11</sup> CFU total; ~10<sup>7</sup> CFU/mL final concentration). The cultures were centrifuged at 5000× g for 10 min, and the cell pellets were resuspended in wort prior to inoculation. After thorough mixing, the wort was fermented at room temperature for two days. Once the pH dropped to approximately 3.3–3.5, the sour wort was boiled for one hour to halt LAB activity, with 28 g of Cascade hop pellets (T-90) (Yakima Classic Premium Hops, Inc., Yakima, WA, USA) added during the final 15 min. The control beer did not undergo the souring process and was transferred directly to the boiling step after mashing.

After cooling to below 30 °C, the wort was transferred to a clean fermentation barrel and inoculated with dry brewing yeast *Saccharomyces cerevisiae* US-05 (~5 g; ~10<sup>6</sup> CFU/mL;

Fermentis, Lille, France). Fermentation proceeded at 20 °C for about a week. On day five, 28 g of Cascade hops was added for dry hopping. Original gravity (OG) was measured prior to fermentation, and final gravity (FG) was recorded post-fermentation using the portable density meter (DMA 35, Anton Paar, Graz, Austria) to assess yeast performance. Alcohol by volume (ABV) was determined using the CDR BeerLab alcohol assay ( $\pm 0.1\%$ ) (Florence, Italy).

Post-fermentation, beer was racked to remove sediments, and glucose (7 g/L) was added to trigger secondary fermentation in bottles for natural carbonation. The bottles were conditioned at 20 °C for one month, then pasteurized at 65 °C for 30 min, and subsequently refrigerated prior to analysis or tasting.

### 2.5. Analysis of Volatile Compounds in Beer Samples

Volatile compounds in beer samples were analyzed using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) analysis ( $\pm 5\%$  relative standard deviation across replicates) (GCMS-QP2010 SE, Shimadzu Corporation, Kyoto, Japan). Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) fibers were used for adsorption, with pre-desorption performed to eliminate potential environmental interferences. Fresh beer samples (5 mL) were placed in 20 mL brown vials, spiked with 1  $\mu$ L of an internal standard (1% *trans*-cinnamaldehyde) [30], sealed and heated at 50 °C. The SPME fibers were exposed to the headspace for 30 min, then thermally desorbed in the GC injector at 280 °C using a split injection mode with a split ratio of 20:1. VOCs were separated using an Rtx-5MS column (30 m  $\times$  0.25 mm  $\times$  0.5  $\mu$ m) with helium as the carrier gas at a flow rate of 1.1 mL/min. The oven temperature was initially held at 40 °C for 2 min, then increased to 140 °C at 10 °C/min, followed by a ramp to 280 °C at 7 °C/min, and held for 3 min. Mass spectrometric detection was performed using electron ionization (EI) at 70 eV. The ion source temperature was set to 260 °C, and the interface temperature was maintained at 280 °C. Data were acquired in scan mode over a mass range of 35–650 *m/z*. The relative concentrations of the identified compounds were determined by comparing their peak areas to that of the internal standard (*trans*-cinnamaldehyde), enabling semi-quantitative analysis of the volatile profile.

### 2.6. Data Analysis

Experimental data were statistically analyzed using one-way ANOVA for independent samples to assess significant differences among treatments ( $N = 3$  and  $n = 3$ ). Where significant effects were found, post hoc Tukey's HSD tests were performed to identify pairwise differences, using IBM SPSS Statistics 20 (IBM, Armonk, NY, USA). For volatile compounds analysis, the peak areas from GC-MS were normalized relative to the internal standard (*trans*-cinnamaldehyde), providing semi-quantitative data. These normalized values were used to construct the data matrix for further analysis. To investigate patterns and associations among volatile compounds, we conducted Principal Component Analysis (PCA) using XLSTAT (Lumivero, Denver, CO, USA). Prior to PCA, the dataset was autoscaled (mean-centered and scaled to unit variance) to ensure comparability between variables. The PCA enabled dimensionality reduction and visualization of the relationships between different beer samples and their associated chemical profiles.

## 3. Results and Discussion

### 3.1. LAB Morphology and Their Identity

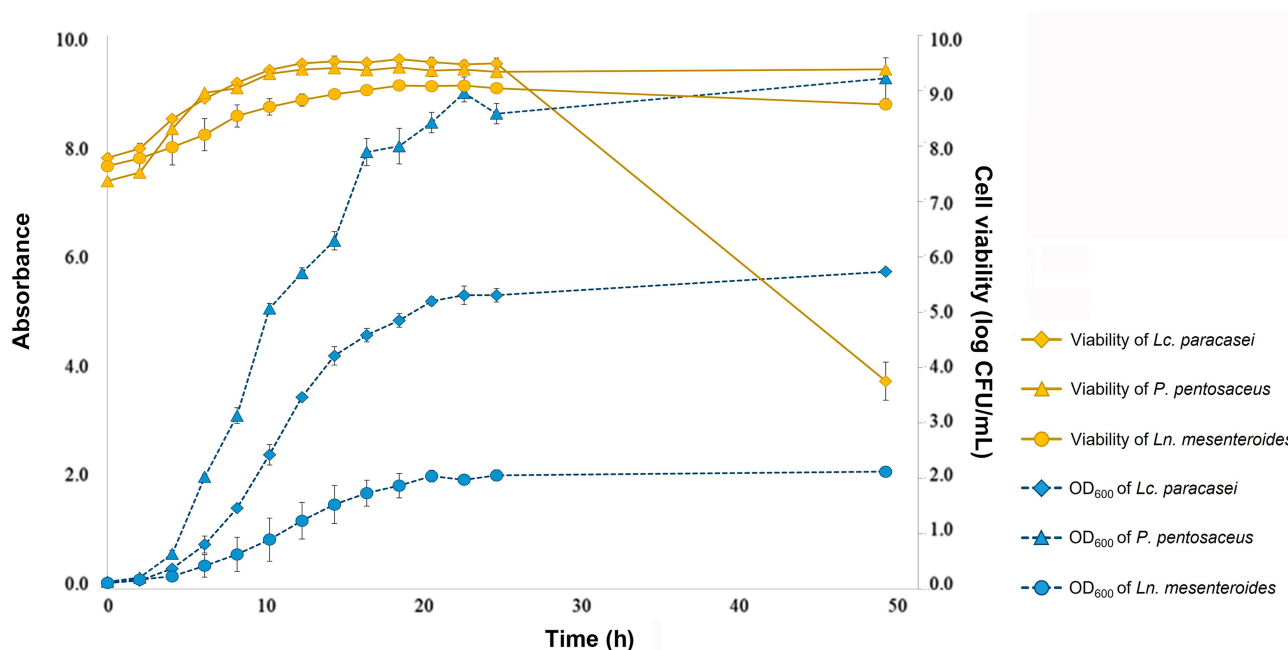
Since the three LAB strains were gifted by Taiwan Ale Beer Co., Ltd., their identities were verified before proceeding with further experiments. *L. paracasei*, *P. pentosaceus*, and *L. mesenteroides* were first cultured on MRS agar plates, where all three species formed

milky white, round, slightly convex colonies with smooth edges (Figure 1, upper panel). Their cellular morphologies were then examined using an inverted fluorescence microscope at 1600 $\times$  magnification. *L. paracasei* appeared as rod-shaped cells approximately 5.0  $\mu\text{m}$  long and 1.0  $\mu\text{m}$  wide (Figure 1, bottom left). *P. pentosaceus* formed oval-shaped diplococci, around 1.2  $\mu\text{m}$  in length and 1.5  $\mu\text{m}$  in width (Figure 1, bottom middle). *L. mesenteroides* presented as cocci arranged in short chains, measuring about 1.2  $\mu\text{m}$  long and 1.0  $\mu\text{m}$  wide (Figure 1, bottom right). These observations confirmed distinct morphological features, supporting their species identification.

To further validate the identities, molecular analysis was conducted. Using bacterial universal primers 27F and 1492R, 16S rRNA gene fragments (~1.5 kb) were amplified, cloned into plasmids, and sequenced. BLAST+ (v2.16.0) analysis against the NCBI database confirmed all three strains as LAB species. The 16S rRNA sequence of *L. paracasei* (1535 bp) showed 100% identity with the *L. paracasei* TCS strain (CP038153.1). *P. pentosaceus* (1542 bp) also showed 100% identity with the *P. pentosaceus* HBUAS63079 strain (OM936151.1). *L. mesenteroides* (1516 bp) displayed 99.93% identity with the *L. mesenteroides* SRCM102735 strain (CP028255.1), differing only at nucleotide positions 627 (A  $\rightarrow$  G) and 1263 (G  $\rightarrow$  A). Together, the morphological and molecular analyses robustly confirmed the identities of the three LAB strains.

### 3.2. Growth Characteristics of LAB

To assess growth characteristics and determine appropriate pitching rates for brewing, the three LAB species were cultured in MRS medium and monitored by optical density (OD) and viable cell counts (log CFU/mL) (Figure 2). All strains reached saturations within 24 h (blue dashed lines), achieving  $\sim 10^9$  CFU/mL at peak viability (yellow solid lines).



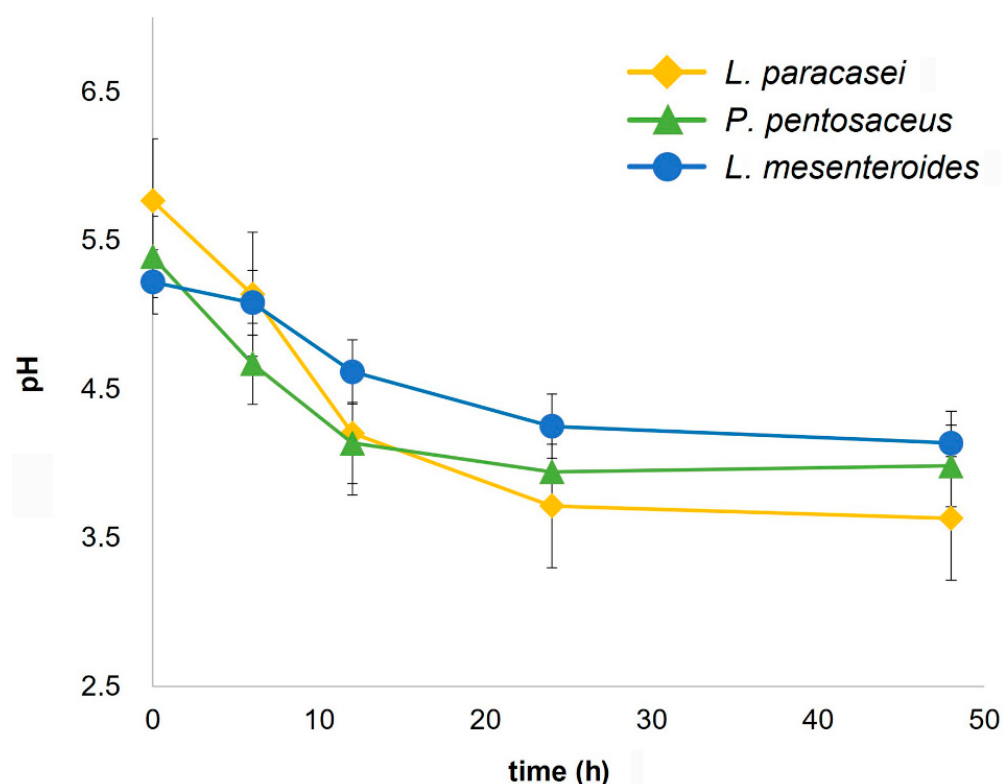
**Figure 2.** Growth curves of lactic acid bacteria. The growth curves of *L. paracasei*, *P. pentosaceus*, and *L. mesenteroides* were monitored over time. Optical density (OD<sub>600</sub>) was measured every 2 h to represent total bacterial concentration (blue dashed line), while viable cell counts were determined by serial dilution and plating, expressed as colony-forming units per milliliter (yellow solid line, log scale). Symbols indicate different species: diamond, *L. paracasei*; triangle, *P. pentosaceus*; circle, *L. mesenteroides*.

The viable cell count of *L. paracasei* peaked at 8 h and then declined sharply to  $10^3$  CFU/mL at 48 h (yellow diamond line), likely due to acid stress or nutrient depletion [31,32]. *P. pentosaceus* showed robust and stable growth, maintaining over  $10^9$  CFU/mL throughout the 48 h (yellow triangle line). a noticeable growth slowdown after 12 h (blue triangle line). *L. mesenteroides* grew more slowly, saturating at 18 h with lower OD but maintained viable counts near  $10^9$  CFU/mL (blue circle line), possibly due to cell aggregation affecting OD readings.

In summary, *P. pentosaceus* demonstrated the best growth stability, while *L. paracasei* should be harvested within 24 h to avoid viability loss. *L. mesenteroides* requires longer cultivation and potentially larger volumes due to slower growth.

### 3.3. Acidification Effects of LAB Species in MRS Medium and Wort

Three LAB species were inoculated into MRS medium to assess acidification. All strains exhibited a rapid decrease in pH within the first 10 h, followed by no statistically significant changes between 24 and 48 h (Figure 3). *L. paracasei* showed the greatest reduction (5.77 to 3.63), followed by *P. pentosaceus* (5.39 to 3.98) and *L. mesenteroides* (5.22 to 4.13).



**Figure 3.** pH changes in lactic acid bacteria cultured in MRS. The graph illustrates the pH changes in lactic acid bacteria cultured in MRS medium over time. The yellow line represents the *L. paracasei*, the green line indicates the *P. pentosaceus*, and the blue line denotes the *L. mesenteroides*.

For kettle souring, wort pH was measured before and after LAB inoculation (Table 1, based on three independent brewing trials  $N = 3$ , each with three replicates  $n = 3$ ). *L. paracasei* dropped pH to 3.26 after two days, while *P. pentosaceus* and *L. mesenteroides* reached 3.41 and 3.50, respectively. A significant pH difference was observed between *L. paracasei* and *L. mesenteroides*, though *P. pentosaceus* was not statistically different from either. Boiling slightly increases pH, likely due to protein and acid volatilization, but this had no impact on fermentation.

**Table 1.** pH, gravity, alcohol content, and microbial parameters of experimental beers fermented with different lactic acid bacteria strains compared to the control beer.

| Characters                 |                      | <i>L. paracasei</i> Beer | <i>P. pentosaceus</i> Beer | <i>L. mesenteroides</i> Beer | Control Beer       |
|----------------------------|----------------------|--------------------------|----------------------------|------------------------------|--------------------|
| pH                         | Water                | 7.22 <sup>a</sup>        | 7.18 <sup>a</sup>          | 7.16 <sup>a</sup>            | 7.26 <sup>a</sup>  |
|                            | Wort                 | 5.67 <sup>a</sup>        | 5.63 <sup>a</sup>          | 5.64 <sup>a</sup>            | 5.64 <sup>a</sup>  |
|                            | Kettle soured        | 3.26 <sup>a</sup>        | 3.41 <sup>ab</sup>         | 3.50 <sup>b</sup>            | NA                 |
|                            | End of boiling       | 3.35 <sup>a</sup>        | 3.51 <sup>ab</sup>         | 3.58 <sup>b</sup>            | 5.41 <sup>c</sup>  |
|                            | Primary fermentation | 3.33 <sup>a</sup>        | 3.43 <sup>ab</sup>         | 3.52 <sup>b</sup>            | 4.19 <sup>c</sup>  |
|                            | Matured              | 3.43 <sup>a</sup>        | 3.48 <sup>a</sup>          | 3.65 <sup>a</sup>            | 4.21 <sup>b</sup>  |
| Original gravity           |                      | 1.059 <sup>a</sup>       | 1.059 <sup>a</sup>         | 1.054 <sup>a</sup>           | 1.057 <sup>a</sup> |
| Final gravity              |                      | 1.017 <sup>a</sup>       | 1.017 <sup>a</sup>         | 1.018 <sup>a</sup>           | 1.013 <sup>a</sup> |
| Alcohol by volume (%)      |                      | 5.60 <sup>a</sup>        | 5.57 <sup>a</sup>          | 4.77 <sup>b</sup>            | 5.79 <sup>a</sup>  |
| Pitching rate log (CFU/mL) |                      | 7.45 <sup>a</sup>        | 6.99 <sup>a</sup>          | 7.07 <sup>a</sup>            | NA                 |

(N = 3, n = 3). Notes: The labels <sup>a, b, c</sup> are used to compare three beer samples. Grouping is based on significant differences (*p* < 0.05). “NA” indicates that the data was not available.

All three beers had an alcohol content around 5%, except *L. mesenteroides* beer (4.77%), likely due to its lower original gravity (1.054 vs. 1.059; Table 1). Since the alcohol content produced by *S. cerevisiae* did not differ significantly between the control and the samples fermented with *L. paracasei* and *P. pentosaceus*, it suggests that the pH variations did not substantially affect the fermentation efficiency of *S. cerevisiae*.

Overall, *L. paracasei* exhibited the fastest acidification, consistent with previous findings that highlight its robust acid-producing capacity under favorable conditions. In contrast, *P. pentosaceus* and *L. mesenteroides* demonstrated slower acidification rates, aligning with their known heterofermentative metabolism and comparatively lower acid tolerance [33,34]. The slight post-boil pH increase did not alter fermentation or alcohol production.

3.4. Volatile Compounds Analysis in Sour IPA

The volatile compounds in beers acidified by *L. paracasei*, *P. pentosaceus*, *L. mesenteroides*, and a control (non-acidified) beer were analyzed. Volatile compounds were identified by matching mass spectra to the NIST 2020 spectral library (National Institute of Standards and Technology), using a similarity threshold greater than 90%. Relative peak areas were calculated using cinnamaldehyde as an internal standard. Among the top 50 peaks in each sample, only 17 esters, 4 alcohols, 4 terpenoids, 2 acids, 4 alkenes (including cyclic alkenes), and 1 phenolic (Table 2) met both criteria. Compounds classified under “Alkenes (including cyclic alkenes)” were grouped based on the presence of one or more carbon–carbon double bonds, following IUPAC nomenclature and supported by data from chemical databases such as PubChem and ChemSpider.

Notable differences emerged between the samples. Beer fermented with *L. paracasei* showed the lowest levels of phenylethyl acetate, ethyl 9-decenoate, phenylethyl alcohol, and citronellol (Table 2). Several compounds, including ethyl dodecanoate, α-terpinol, citronellol, and geraniol, were common across LAB-fermented beers but differed significantly from the control.

These variations highlight the distinct impacts of different LAB species on the volatile profiles of sour IPAs, suggesting that each strain contributes unique aromatic and flavor characteristics.

**Table 2.** Volatile compounds in three beer samples and a beer sample without added lactic acid bacteria.

| Volatile Compounds                        | Odor                          | <i>L. paracasei</i>        | <i>P. pentosaceus</i>       | <i>L. mesenteroides</i>     | Control Beer                |
|-------------------------------------------|-------------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|
| <b>Esters</b>                             |                               |                            |                             |                             |                             |
| ethyl acetate                             | fruity, nail polish           | 0.174 ± 0.077 <sup>a</sup> | 0.206 ± 0.070 <sup>a</sup>  | 0.180 ± 0.034 <sup>a</sup>  | 0.238 ± 0.067 <sup>a</sup>  |
| isobutyl acetate                          | banana                        | 0.033 ± 0.014 <sup>a</sup> | 0.044 ± 0.007 <sup>a</sup>  | 0.050 ± 0.022 <sup>a</sup>  | 0.039 ± 0.002 <sup>a</sup>  |
| ethyl butanoate                           | strawberry, lactic            | 0.047 ± 0.011 <sup>a</sup> | 0.068 ± 0.025 <sup>a</sup>  | 0.053 ± 0.013 <sup>a</sup>  | 0.043 ± 0.012 <sup>a</sup>  |
| isoamyl acetate                           | banana                        | 0.715 ± 0.321 <sup>a</sup> | 0.760 ± 0.387 <sup>a</sup>  | 0.563 ± 0.085 <sup>a</sup>  | 1.008 ± 0.287 <sup>a</sup>  |
| 2-methylbutyl acetate                     | fruity, banana                | 0.081 ± 0.014 <sup>a</sup> | 0.034 ± 0.059 <sup>a</sup>  | 0.049 ± 0.044 <sup>a</sup>  | 0.105 ± 0.018 <sup>a</sup>  |
| isobutyl isobutyrate                      | Pineapple                     | 0.208 ± 0.034 <sup>a</sup> | 0.179 ± 0.160 <sup>a</sup>  | 0.163 ± 0.151 <sup>a</sup>  | 0.178 ± 0.012 <sup>a</sup>  |
| 2-pentanol propanoate                     | apple                         | 0.020 ± 0.034 <sup>b</sup> | 0.082 ± 0.015 <sup>a</sup>  | 0.090 ± 0.015 <sup>a</sup>  | 0.019 ± 0.032 <sup>b</sup>  |
| ethyl hexanoate                           | apple peel, overripe fruit    | 0.440 ± 0.148 <sup>a</sup> | 0.565 ± 0.163 <sup>a</sup>  | 0.337 ± 0.052 <sup>a</sup>  | 0.548 ± 0.069 <sup>a</sup>  |
| isoamyl isobutyrate                       | banana                        | 0.121 ± 0.026 <sup>a</sup> | 0.131 ± 0.054 <sup>a</sup>  | 0.144 ± 0.054 <sup>a</sup>  | 0.107 ± 0.014 <sup>a</sup>  |
| 2-methylbutyl isobutyrate                 | earthy                        | 0.571 ± 0.114 <sup>a</sup> | 0.549 ± 0.362 <sup>a</sup>  | 0.568 ± 0.358 <sup>a</sup>  | 0.489 ± 0.050 <sup>a</sup>  |
| ethyl heptanoate                          | fruity, pineapple             | 0.055 ± 0.007 <sup>a</sup> | 0.050 ± 0.047 <sup>a</sup>  | 0.033 ± 0.057 <sup>a</sup>  | 0.018 ± 0.031 <sup>a</sup>  |
| ethyl octanoate                           | pear, apricot                 | 2.847 ± 0.663 <sup>a</sup> | 3.586 ± 0.640 <sup>a</sup>  | 2.347 ± 0.295 <sup>a</sup>  | 3.133 ± 0.276 <sup>a</sup>  |
| phenylethyl acetate                       | fruity, honey, rose           | 0.249 ± 0.025 <sup>b</sup> | 0.466 ± 0.050 <sup>a</sup>  | 0.374 ± 0.067 <sup>ab</sup> | 0.377 ± 0.072 <sup>ab</sup> |
| methyl geranate                           | sweet, candy                  | 0.022 ± 0.039 <sup>a</sup> | 0.117 ± 0.041 <sup>a</sup>  | 0.128 ± 0.068 <sup>a</sup>  | 0.073 ± 0.016 <sup>a</sup>  |
| ethyl 9-decenoate                         | fruity                        | 0.053 ± 0.017 <sup>b</sup> | 0.185 ± 0.176 <sup>ab</sup> | 0.127 ± 0.072 <sup>ab</sup> | 0.357 ± 0.100 <sup>a</sup>  |
| ethyl decanoate                           | floral, soap                  | 0.731 ± 0.172 <sup>a</sup> | 0.837 ± 0.355 <sup>a</sup>  | 0.385 ± 0.071 <sup>a</sup>  | 0.369 ± 0.177 <sup>a</sup>  |
| ethyl dodecanoate                         | floral, fruity, buttery, pear | 0.096 ± 0.031 <sup>a</sup> | 0.124 ± 0.039 <sup>a</sup>  | 0.065 ± 0.014 <sup>a</sup>  | ND <sup>b</sup>             |
| <b>Alcohols</b>                           |                               |                            |                             |                             |                             |
| isobutanol                                | fusel, alcohol                | 0.098 ± 0.009 <sup>a</sup> | 0.144 ± 0.042 <sup>a</sup>  | 0.114 ± 0.013 <sup>a</sup>  | 0.152 ± 0.017 <sup>a</sup>  |
| isoamyl alcohol                           | mild, choking alcohol         | 0.902 ± 0.118 <sup>a</sup> | 1.009 ± 0.218 <sup>a</sup>  | 0.856 ± 0.116 <sup>a</sup>  | 1.070 ± 0.127 <sup>a</sup>  |
| 2-methylbutanol                           | wine, onion                   | 0.307 ± 0.029 <sup>a</sup> | 0.311 ± 0.070 <sup>a</sup>  | 0.242 ± 0.044 <sup>a</sup>  | 0.409 ± 0.040 <sup>a</sup>  |
| phenylethyl alcohol                       | rose                          | 0.672 ± 0.159 <sup>b</sup> | 1.410 ± 0.436 <sup>a</sup>  | 1.048 ± 0.160 <sup>ab</sup> | 0.835 ± 0.067 <sup>ab</sup> |
| <b>Terpenoids</b>                         |                               |                            |                             |                             |                             |
| linalool                                  | floral, citrus                | 0.469 ± 0.101 <sup>a</sup> | 0.568 ± 0.120 <sup>a</sup>  | 0.589 ± 0.056 <sup>a</sup>  | 0.421 ± 0.071 <sup>a</sup>  |
| α-terpineol                               | lilac, spicy                  | 0.146 ± 0.034 <sup>a</sup> | 0.157 ± 0.043 <sup>a</sup>  | 0.142 ± 0.005 <sup>a</sup>  | 0.045 ± 0.016 <sup>b</sup>  |
| citronellol                               | citrus                        | 0.119 ± 0.013 <sup>c</sup> | 0.177 ± 0.053 <sup>bc</sup> | 0.223 ± 0.018 <sup>b</sup>  | 0.245 ± 0.023 <sup>a</sup>  |
| geraniol                                  | floral, sweet rose, citrus    | 0.238 ± 0.050 <sup>a</sup> | 0.257 ± 0.056 <sup>a</sup>  | 0.242 ± 0.011 <sup>a</sup>  | 0.219 ± 0.065 <sup>b</sup>  |
| <b>Acids</b>                              |                               |                            |                             |                             |                             |
| octanoic acid                             | pungent, mild                 | 0.392 ± 0.028 <sup>a</sup> | 0.410 ± 0.060 <sup>a</sup>  | 0.297 ± 0.044 <sup>a</sup>  | 0.299 ± 0.105 <sup>a</sup>  |
| decanoic acid                             | rancid, unpleasant            | 0.087 ± 0.013 <sup>a</sup> | 0.104 ± 0.034 <sup>a</sup>  | 0.055 ± 0.049 <sup>a</sup>  | ND <sup>b</sup>             |
| <b>Alkenes (including cyclic alkenes)</b> |                               |                            |                             |                             |                             |
| myrcene                                   | woody                         | 0.756 ± 0.064 <sup>a</sup> | 1.230 ± 0.955 <sup>a</sup>  | 0.867 ± 0.146 <sup>a</sup>  | 0.667 ± 0.080 <sup>a</sup>  |
| styrene                                   | solvently, rubbery            | 0.070 ± 0.061 <sup>a</sup> | 0.023 ± 0.040 <sup>a</sup>  | 0.078 ± 0.071 <sup>a</sup>  | 0.041 ± 0.072 <sup>a</sup>  |
| trans-β-farnesene                         | woody, citrus, herbal         | 0.055 ± 0.009 <sup>a</sup> | 0.047 ± 0.044 <sup>a</sup>  | 0.066 ± 0.010 <sup>a</sup>  | 0.052 ± 0.017 <sup>a</sup>  |
| humulene                                  | spicy, woody                  | 0.075 ± 0.006 <sup>a</sup> | 0.192 ± 0.157 <sup>a</sup>  | 0.197 ± 0.127 <sup>a</sup>  | 0.129 ± 0.035 <sup>a</sup>  |
| <b>Phenolics</b>                          |                               |                            |                             |                             |                             |
| butylhydroxytoluene                       | musty                         | 0.307 ± 0.249 <sup>a</sup> | 0.275 ± 0.239 <sup>a</sup>  | 0.479 ± 0.141 <sup>a</sup>  | 0.113 ± 0.184 <sup>a</sup>  |

(N = 3, n = 3). Notes: 1. Data represent the relative area, with cinnamaldehyde (internal standard) as the reference (ratio of 1). 2. The labels <sup>a</sup>, <sup>b</sup>, and <sup>c</sup> are used to compare three beer samples and a beer without lactic acid bacteria. Grouping is based on significant differences ( $p < 0.05$ ). 3. “ND” indicates that the compound was not detected in the sample.

### 3.5. Influence of LAB Species on Volatile Compounds Formation

The comparison of volatile compounds in the three sour IPA beer samples demonstrated that different LAB species—*L. paracasei*, *P. pentosaceus*, and *L. mesenteroides*—significantly influenced the formation and transformation of volatiles during fermentation. Among esters, 2-pentanol propanoate was notably higher in *P. pentosaceus* and *L. mesenteroides* beers (Table 2), contributing an apple-like aroma. Another key ester, phenylethyl acetate, known for its fruity, honey, and rose-like notes [35], was highest in *P. pentosaceus* beer, which also exhibited the highest levels of phenylethyl alcohol, reinforcing a floral scent. Given their low odor thresholds (250 ppb for phenylethyl acetate and 10 ppm for phenylethyl alcohol) [36,37], even small amounts can strongly enhance perception, likely making *P. pentosaceus* beer more aromatic.

Ethyl 9-decenoate, a fruity ester [38], was lower in all LAB-inoculated beers compared to the control, suggesting that LAB activity affected its formation. The reduction may be due to decreased availability of 9-decenoic acid, typically derived from decanoic acid via Δ9-desaturase [39]. As a speculation, we proposed that LAB fermentation may produce

heat-stable compounds that persist through the post-souring wort boiling process and potentially inhibit  $\Delta 9$ -desaturase activity. Alternatively, the low pH conditions might suppress esterification reactions. Either mechanism could contribute to the observed accumulation of decanoic acid (Table 2). Consequently, ethyl decanoate—associated with soapy or floral scents [40]—was slightly elevated in the LAB beers. Additionally, ethyl dodecanoate, linked to floral, fruity, and buttery notes [38,41], was detected in all LAB-inoculated beers but absent in the control, potentially contributing to a lighter and more refreshing aroma profile.

These findings highlight the distinct influence of LAB species on the volatile profile of sour IPAs, shaping their aroma characteristics.

### 3.6. Terpenoids and Their Impact on Beer Flavor

Terpenoid alcohols such as geraniol,  $\alpha$ -terpinol, and citronellol play a key role in beer flavor, enhancing fruity and floral aromas. These compounds originate from hop terpenes, like myrcene and humulene, and are biotransformed by yeast during fermentation [42].

Geraniol, known for its rose and lemon-like aroma, was detected in all samples (Table 2), but was lower in the non-LAB beer, likely due to reduced yeast biotransformation. With its low odor threshold (~30 ppb), even small amounts significantly impact aroma [37]. Citronellol, a citrus-scented terpenoid, was also found in all beers but at lower levels in LAB-inoculated samples, suggesting that lower pH may hinder the yeast's ability to convert geraniol into citronellol [43]. Notably, *L. paracasei* beer (pH 3.38) had less citronellol than *L. mesenteroides* beer (pH 3.58), reinforcing the role of acidity in yeast metabolism.

Similarly,  $\alpha$ -terpinol, a spicy-scented terpenoid with a 250 ppb odor threshold [44,45], showed pH-dependent variations. Derived from linalool, it highlights the intricate interplay between yeast metabolism and environmental acidity in shaping aroma.

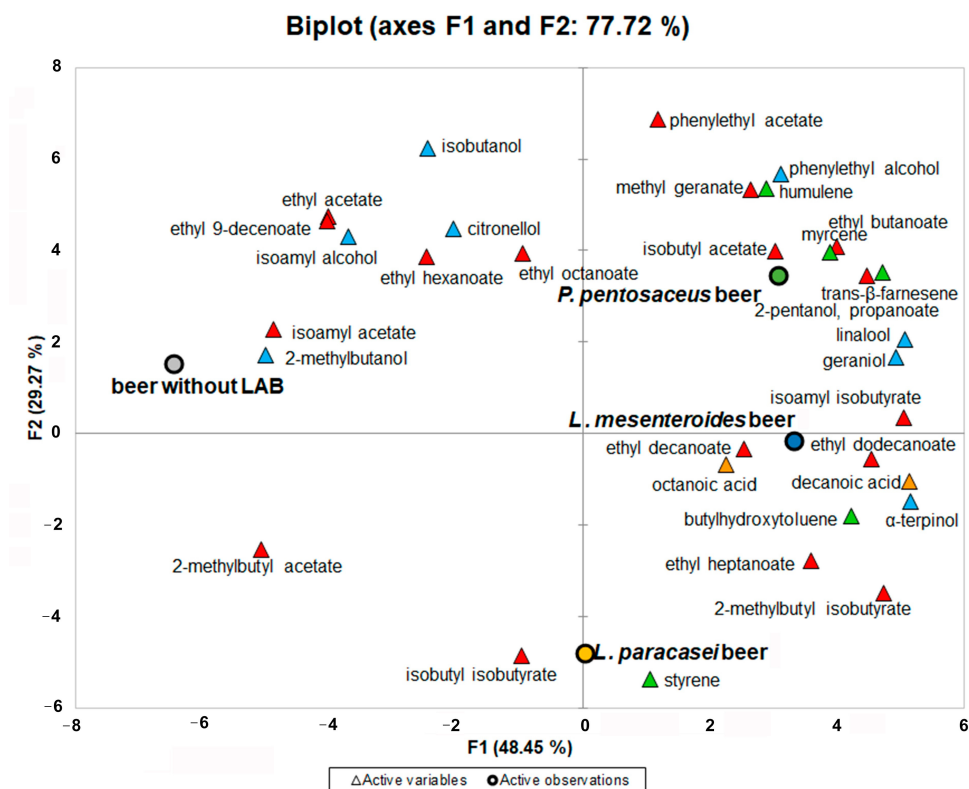
In summary, the conversion of terpenoids was influenced by LAB fermentation, with pH affecting yeast-driven biotransformations. These findings emphasize the importance of both yeast activity and acidity in defining the aromatic complexity of sour IPAs.

### 3.7. Principal Component Analysis (PCA) of Volatile Compounds

The PCA of volatile compounds (Figure 4) reveals distinct flavor differences among the three LAB-inoculated beers and the control. The first two principal components (F1 and F2) explain 77.72% of total variance (48.45% and 29.27%, respectively), indicating that the detected volatiles effectively capture the key differences in flavor profiles.

The clear separation between LAB-inoculated and control beers highlights the significant impact of LAB fermentation on flavors. *L. mesenteroides* beer is closer to *L. paracasei* beer than to *P. pentosaceus* beer, suggesting greater similarity in their volatile profiles. Notably, phenylethyl alcohol—a rose-scented compound with well-known floral and aromatic qualities—is prominently associated with *P. pentosaceus* beer, indicating a distinct metabolic contribution to its complex aroma profile. This observation is consistent with findings in sourdough and fermented vegetable studies [46,47].

Overall, PCA confirms that LAB species significantly influence beer aroma, with *P. pentosaceus* beer exhibiting the most complex profile, while *L. mesenteroides* and *L. paracasei* beers share more similarities. In addition to quantitative differences, the qualitative nature of volatile compounds—such as the presence of floral terpenoids or buttery diacetyl precursors—highlights each strain's unique contribution to beer style differentiation. While this study standardized pH, temperature, and wort composition to isolate strain-specific effects, LAB performance is strongly influenced by these parameters. Future investigations should systematically vary these process conditions to optimize souring efficiency and flavor outcomes for industrial applications.



**Figure 4.** Principal component analysis (PCA) of volatile compounds in three beer samples. PCA was conducted on the relative content of volatile compounds in three sour beer samples and one beer sample without added lactic acid bacteria. Circles represent the samples, while four different patterned triangles denote the volatile compounds identified via gas chromatography-mass spectrometry (GC-MS).

#### 4. Conclusions

This study demonstrated that three LAB species contribute distinctively to sour beer production through differences in growth, acidification, and volatile compound profiles. *P. pentosaceus* exhibited the fastest and most stable growth and enriched fruity and floral notes, *L. paracasei* produced the lowest pH and most intense sourness, and *L. mesenteroides* resulted in milder acidity and a balanced flavor. These strain-specific traits can be strategically applied to create fruit-forward, bold sour, or refreshing beer styles, offering brewers targeted options for flavor innovation.

Although promising, this work was limited to a single beer formulation. Future studies should expand to pilot-scale brewing, explore varied malt and hop compositions, and evaluate shelf stability and flavor retention over time to further validate the commercial potential of each LAB strain.

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