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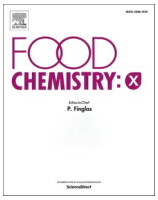

Lactiplantibacillus plantarum exerts strain-specific effects on malolactic fermentation, antioxidant activity, and aroma profile of apple cider

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A R T I C L E I N F O

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A B S T R A C T

This study aimed to investigate the impact of different strains of *Lactiplantibacillus plantarum* on malolactic fermentation (MLF), antioxidant activity, and aroma of ciders. A commercial strain of *Saccharomyces cerevisiae* and six indigenous *L. plantarum* strains were co-inoculated into apple juice to induce simultaneous alcoholic fermentation (AF) and MLF. The findings indicated that despite belonging to the same species, the different L. *plantarum* strains significantly differed ($p < 0.05$) in terms of antioxidant activity and aroma compounds in the ciders. MLF induced by L. *plantarum* resulted in the substantial consumption of malic acid and increased levels of lactic acid in the ciders, with strain-specific effects observed, particularly with L. *plantarum* SCFF284. In addition, ciders produced from mixed fermentations exhibited higher levels of antioxidant activity than those from pure *S. cerevisiae* fermentation ($p < 0.05$), especially for LAM284. Furthermore, ciders produced from mixed fermentations exhibited higher levels of aroma compounds, such as ethyl acetate and isoamyl alcohol, and also received higher sensory scores compared to ciders produced through pure *S. cerevisiae* fermentation ($p < 0.05$). These results highlight the effectiveness of MLF induced by L. *plantarum* in enhancing the antioxidant activity and aroma profile of ciders.

1. Introduction

Malolactic fermentation (MLF), which usually takes place during or following alcoholic fermentation (AF), involves the conversion of L-malic acid to L-lactic acid, a highly desirable process in fruit wines (Jiang et al., 2018). MLF is renowned for its ability to enhance fruit wine quality by reducing acidity, improving microbial stability, generating favorable flavor compounds, and creating a smoother mouthfeel. Moreover, MLF can further transform aroma precursors in fruit wine into free-form volatile compounds (Knoll et al., 2011). However, MLF is not always effectively achieved, and the fermentation process may take place over weeks without obtaining satisfactory results (Agouridis et al., 2005). Prolonged fermentation time can hinder vinification efficiency and increase the risk of off-flavors caused by spoilage microorganisms (Sumby et al., 2019). Therefore, the reliable and efficient attainment of MLF is essential for enhancing the quality of fruit wines.

Previous research has highlighted the presence of various species and strains of lactic acid bacteria (LAB) in spontaneous MLF, although *Oenococcus oeni* has often been identified as the dominant species in many MLF studies (Lucio et al., 2017). Within the LAB species, *Lactiplantibacillus plantarum* stands out as a highly promising candidate for the challenging vinification environment of fruit wines. *L. plantarum* exhibits notable resistance to the ethanol concentration up to 14% (*v*/v) and pH values as low as 3.2, making it well-suited for fruit wine production (Brizuela et al., 2019; Iorizzo et al., 2016). In addition, *L. plantarum* strains possess a more diverse range of enzyme profiles than *O. oeni* strains, particularly with respect to aroma-modifying enzymes such as β-glucosidase and phenolic acid decarboxylase (du Toit et al., 2011). Moreover, *L. plantarum* offers several advantageous biological properties, including rapid growth, prevention of undesirable

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compound formation, and a wide array of enzymes that contribute to the aroma and color of fruit wines. These attributes position L. *plantarum* strains as suitable candidates for MLF starter cultures (Lucio et al., 2017).

Yanyuan Fuji apples, cultivated in Yanyuan County of Sichuan Province and harvested in December, possess distinct characteristics that make them highly suitable for cider production. These apples thrive in a natural ecological environment with abundant sunlight exposure, resulting in a higher concentration of polyphenols, sugars, and a wide range of flavor compounds. However, in cider production, spontaneous MLF involving various LAB strains presents challenges in predictability and can lead to fermentation delays. Prolonged MLF and residual nutrients provide an optimal environment for the growth of spoilage microorganisms (Sumby et al., 2019), which can produce undesirable biogenic amines and off-flavors (Diez-Ozaeta et al., 2021). A simultaneous inoculation approach has been employed to induce MLF, as bacteria gradually acclimate to the harsh brewing conditions (Jussier et al., 2006). Successful co-fermentation has been demonstrated in apple cider production, highlighting the efficacy of this approach (Hu et al., 2023). Consequently, the simultaneous inoculation of yeast and bacteria into apple juice serves as a reliable strategy to minimize the risk of spoilage microorganisms during this crucial stage of cider production.

Previously, we conducted a study investigating the role of L. *plantarum* in the biotransformation of malic acid to lactic acid in apple ciders (Chen et al., 2023). Building upon that research, the primary objective of this study was to assess the capability of six indigenous L. *plantarum* strains to efficiently and reliably perform MLF during the production of apple ciders. In this lab-scale vinification, a simultaneous inoculation of *S. cerevisiae* and L. *plantarum* was employed using Yanyuan Fuji apples. The impact of different L. *plantarum* strains on MLF efficiency, antioxidant activity, and aroma generation in the final ciders was thoroughly examined and compared. The outcomes of the investigation not only enhance our theoretical understanding but also provide practical insights for optimizing cider processing and improving overall quality.

2. Materials and methods

2.1. S. cerevisiae and L. plantarum strains and culture media

Seven microbial strains were used in this study: the commercial wine strain *S. cerevisiae* SY (Angel Yeast Co., Ltd., Yichang, China) and six L. *plantarum* strains (SCFF19, SCFF134, SCFF173, SCFF180, SCFF185, SCFF284). The yeast strain was cultivated on yeast extract-peptone-dextrose (YPD) medium at 28 °C. The L. *plantarum* strains were cultured on de Man-Rogosa-Sharpe (MRS) medium at 37 °C. All L. *plantarum* strains used in this study were sourced from the Culture Collection of Food Microorganisms of Sichuan University of Science and Engineering (Yibin, China). These strains were carefully selected from various samples, including apples, pickles, and koji.

2.2. Fermentation design

For this study, Yanyuan Fuji apples used were ordered from the supermarket in Yanyuan County in December 2022. The apple cider fermentation protocol utilized in our previous research was employed (Hu et al., 2023). Seven fermentation treatments were conducted as follows: (1) AF without MLF, performed using only the commercial strain *S. cerevisiae* SY (ASY); (2) simultaneous AF and MLF through co-inoculation with *S. cerevisiae* SY and L. *plantarum* SCFF19 (LAM19); (3) simultaneous AF and MLF through co-inoculation with *S. cerevisiae* SY and *L. plantarum* SCFF134 (LAM134); (4) simultaneous AF and MLF through co-inoculation with *S. cerevisiae* SY and *L. plantarum* SCFF173 (LAM173); (5) simultaneous AF and MLF through co-inoculation with *S. cerevisiae* SY and *L. plantarum* SCFF180 (LAM180); (6) simultaneous AF and MLF through co-inoculation with *S. cerevisiae* SY and *L. plantarum* SCFF185 (LAM185); and (7) simultaneous AF and MLF through co-inoculation with *S. cerevisiae* SY and *L. plantarum* SCFF284 (LAM284). The sugar content of the apple juice was adjusted to 20°Brix by the addition of sucrose. Apple juice in each treatment was pasteurized at 95 °C for 5 min in a conical flask and subsequently cooled down to room temperature before inoculation. The inoculation ratio of *S. cerevisiae* and L. *plantarum* in the simultaneous AF and MLF treatments was 1:1. Each treatment involved the inoculation of all microbial strains at a final density of 10^7 CFU/mL in 1000 mL apple juice. The AF or AF and MLF treatments in this study were conducted at 20 °C in darkness for 16 days. After fermentation, the ciders were collected by centrifugation at 7000*g* for 10 min (at 4 °C), and the resulting supernatants were stored at −20 °C for further analysis.

2.3. Malic acid and lactic acid analysis through HPLC

The malolactic ability of six L. *plantarum* strains was evaluated by measuring the consumption of malic acid and the synthesis of lactic acid in the cider samples after fermentation using High-Performance Liquid Chromatography (HPLC), as described by the previous study (Wang et al., 2018). The malic acid and lactic acid standards used in the analysis were of analytical grade (Shanghai Yuanye Bio-Technology Co., Ltd). Prior to the analysis, samples were filtered using a 0.45 μm filter membrane, and an injection volume of 20 μL was used. The concentrations of malic acid and lactic acid were determined utilizing an Ultimate® AQ-C18 column (250 × 4.6 mm i.d., 5 μm, Welch, Shanghai, China) connected to SPD-M20A detector (Shimadzu, Kyoto, Japan) at 210 nm. The eluent consisted of a mixture of 98% potassium dihydrogen phosphate (*v*/v, 0.02 M, pH 2.5) and 2% methanol (*v*/v) and flowed at a constant rate of 1 mL/min. All analyses were performed in triplicates. The analytes were identified and quantified based on the retention time of the standard solutions and the standard curves.

2.4. Basic physicochemical analysis

A comprehensive physicochemical analysis of the apple juice and cider samples was conducted following a previously established method (Wei et al., 2020) with slight modifications. The pH value of the samples was measured using a pH meter (PH-100, Lichen, Shanghai, China). The total acidity of the apple juice and ciders was determined by employing an acid-base titration technique with 0.1 mol/L sodium hydroxide. The reducing sugar content was determined through the use of 3,5-Dinitrosalicylic acid, following the general analysis method outlined in the national standard of China (GB/T 15038–2006). The alcoholic content in the cider samples was determined following the corresponding method from the national standard of China (GB 5009.225–2016).

2.5. S. cerevisiae and L. plantarum cell counts

Prior to quantifying *S. cerevisiae* and *L. plantarum* cells, all samples were serially diluted in sterile 0.85% (*w/v*) NaCl solutions. For the quantification of *S. cerevisiae* cells, a 0.1-mL aliquot of each dilution was plated onto PDA plates and incubated at 28 °C for 24 h to allow the formation of visible colonies (Yu et al., 2022). A similar approach was employed for quantifying L. *plantarum* cells on MRS agar plates supplemented with 0.2 g/L of nystatin. The plates were cultured under anaerobic conditions at 37 °C for 48 h. The concentration of both *S. cerevisiae* and *L. plantarum* cells was expressed in colony-forming units per milliliter (CFU/mL).

2.6. Determination of antioxidant activity

The antioxidant activities of apple juice and cider samples were assessed by quantifying the total phenolic content, total antioxidant activity, and DPPH-free radical superoxide anion-reducing power. These measurements were conducted using commercial assay kits (Sangon Biotech Co. Ltd., China).

2.7. Aromatic compound analysis through HS-SPME/GC–MS

The aroma compounds in apple juice and cider samples were extracted using Headspace Solid-Phase Microextraction (HS-SPME) and subsequently analyzed using Gas Chromatography-Mass Spectrometry (GC–MS), following a previously published method with slight modifications (Zhao et al., 2022). The samples for analysis were prepared by combining 2 mL of apple juice or cider, 6 mL of milli-Q water, 2 g of sodium chloride, and 20 μL of 2-octanol (used as an internal standard) in 20 mL headspace vials. The sample vials were then incubated at 40 °C for 15 min, subjected to a 30-min extraction process, and subsequently analyzed using a GC–MS system (Shimadzu, Kyoto, Japan) equipped with an Agilent 5975 MS detector and a DB-Wax column (60 m × 0.25 mm i.d., 0.25 μm film thickness). Desorption of the analyte solution took place at 230 °C for 5 min in the injection port, with a helium gas flow rate of 1.5 mL/min. The GC was programmed as follows: an initial temperature of 40 °C held for 3 min, followed by a linear increase to 160 °C at a rate of 4 °C/min, and then further increased to 220 °C at a rate of 7 °C/min, where it was maintained for 8 min. Mass spectra were obtained using the electron impact mode (EI) with an energy of 70 eV, scanning over the range of *m/z* 35–350 amu. The MS transfer line and ion source temperatures were maintained at 220 °C and 200 °C, respectively. All analyses were performed in triplicate, and volatile substances were semi-quantitatively measured based on the presence of the internal standard.

2.8. Sensory analysis

Sensory analysis was performed following a modified protocol based on the study by Yang et al. (Yang et al., 2022). The sensory properties of finished apple ciders were evaluated and scored by 15 trained panelists (eight women and seven men). Cider quality was evaluated by six attributes: fruitiness, flavor, sweetness, sourness, bitterness, and overall acceptability. The evaluation was conducted using a 9-point hedonic scale, where a score of one indicated the lowest acceptability and nine indicated the highest. The sensory properties of all the finished ciders were determined by calculating the average scores for these six characteristics.

2.9. Statistical analysis

Each experiment was performed in triplicate, and the analysis results were presented as means ± standard deviation (SD). Statistical significance among different groups was determined through Duncan's test ($p < 0.05$) for multiple comparisons using SPSS software version 26. Hierarchical cluster analysis and principal component analysis (PCA) were conducted using Origin 9.0 software (Hampton, Massachusetts, USA).

3. Results and discussion

3.1. Malic acid consumption and lactic acid synthesis

MLF is pivotal in fruit wine fermentation, contributing to biodeacidification, microbial stability, and aromatic complexity (Iorizzo et al., 2016). In this study, the degradation of malic acid and production of lactic acid during apple cider fermentation were determined using HPLC. As illustrated in Fig. 1, the malic acid content in each treatment group exhibited a significant reduction after fermentation. Notably, the mixed microbial fermentation groups (LAM) displayed notably higher malic acid consumption compared to single fermentation (ASY). Furthermore, a considerable increase in lactic acid levels was observed after fermentation. Specifically, the synthesis of lactic acid in the mixed microbial fermentation treatments (LAM) was significantly greater than that in the single fermentation treatment (ASY) (3.26 mg/L). These results align with previous findings (Brizuela et al., 2019), indicating the robust biological acid-reduction capacity of L. *plantarum*. The significant reduction in malic acid and synthesis of lactic acid during the co-fermentation of apple ciders can be attributed to MLF. Moreover, different L. *plantarum* strains exhibited distinct rates of malic acid consumption and lactic acid synthesis. Notably, the LAM284 treatment group demonstrated the highest percentage of malic acid consumption (87.95%) and the highest level of lactic acid (9.83 mg/L), suggesting L. *plantarum* SCFF284 as a promising candidate strain for acidity reduction during MLF.

3.2. Changes in physicochemical parameters

Fig. 2 depicts the dynamic changes in the fundamental

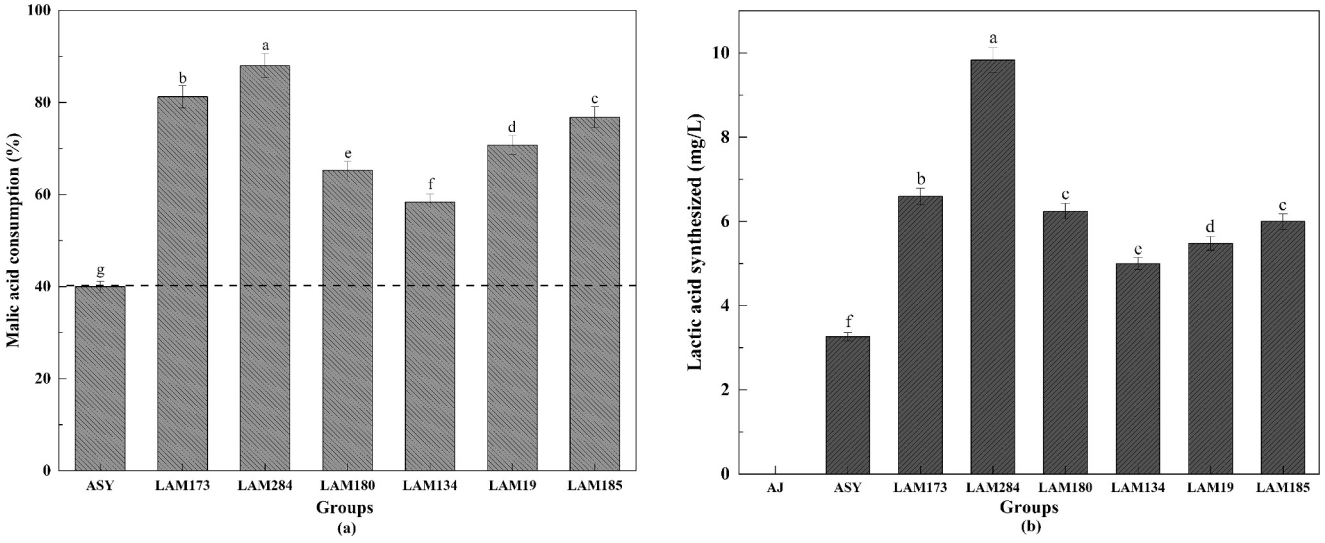


Fig. 1. Consumption percentage of malic acid (a) and level of lactic acid synthesized (b) in apple juice and ciders fermented by single-culture and mixed-cultures. AJ: apple juice without inoculation, ASY: apple cider inoculated with *S. cerevisiae* SY, LAM173: apple cider co-inoculated with *S. cerevisiae* SY and L. *plantarum* SCFF173, LAM284: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF284, LAM180: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF180, LAM134: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF134, LAM19: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF19, LAM185: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF185. Data with different letters (a, b, c, d, e, f, g) within each group are significantly different ($p < 0.05$).

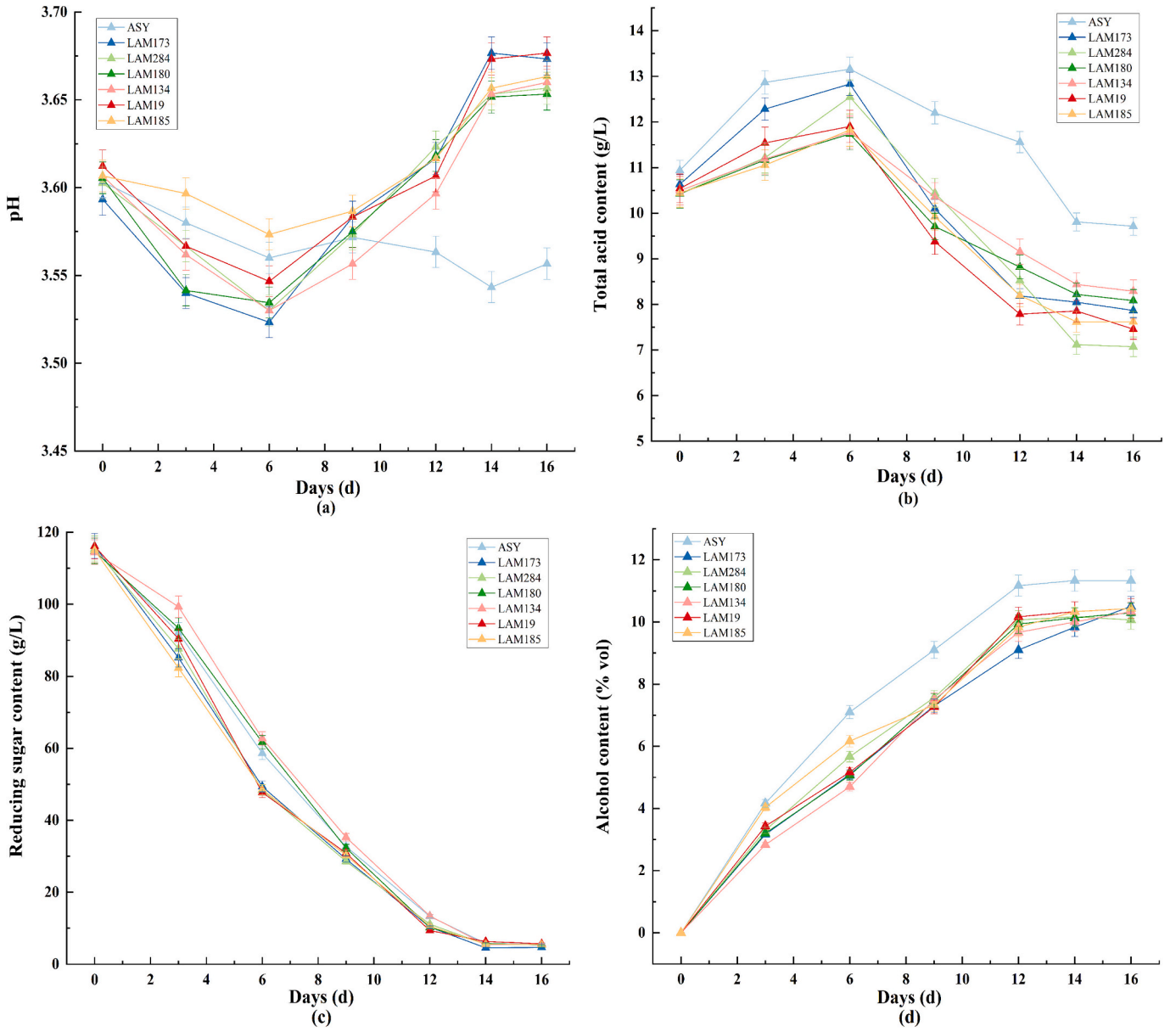


Fig. 2. Dynamic changes of the pH (a), total acidity (b), reducing sugars (c), and alcohol content (d) in apple cider fermented with a single-culture and mixed cultures. ASY: apple cider inoculated with *S. cerevisiae* SY, LAM173: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF173, LAM284: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF284, LAM180: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF180, LAM134: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF134, LAM19: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF19, LAM185: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF185.

physicochemical parameters of ciders. Acidity, a crucial attribute contributing to the distinct taste and freshness of fruit wines, plays a vital role (Braga et al., 2013). Following AF, the pH of ciders fermented solely with yeast (ASY) decreased, while ciders that underwent MLF experienced an increase in pH due to L-malic acid decarboxylation. This finding aligns with previous research (Balmaseda et al., 2021). As presented in Fig. 2b, the total acidity of all ciders decreased significantly, particularly those that underwent MLF, which is consistent with earlier findings (Li, Bi, et al., 2022; Li, Zhang, et al., 2022). Moreover, the total acidity among MLF ciders varied significantly, corresponding to the different strains of L. *plantarum* utilized. The total acidity for the fermentation involving L. *plantarum* strains ranged from 7.07 to 8.08 g/L, which was significantly lower than that of ciders fermented with commercial yeast (9.71 g/L). These results indicate the acid-reducing capability of L. *plantarum*, confirming the findings of a previous study (Devi et al., 2022).

Reducing sugars and alcohol content are important indicators for the fruit wine fermentation process. The continuous consumption of reducing sugars provides both energy and raw materials for microorganisms. Fig. 2c illustrates a consistent decreasing trend in the reducing sugar content during each fermentation treatment, eventually stabilizing. The rate of sugar consumption was higher in the mixed microbial fermentation group (LAM) compared to the single fermentation group (ASY). However, there were no significant differences in the reducing sugar contents among the various ciders at the end of fermentation. Fig. 2d demonstrates an increase in alcohol content across all ciders as reducing sugars were consumed, eventually stabilizing after day 12 of fermentation. In comparison to ciders fermented with a single yeast (ASY) that had an alcohol content of 11.33%, the alcohol content of the mixed-culture fermented ciders ranged from 10.07% to 10.50%, suggesting that L. *plantarum* might also consume a certain amount of sugars to support its own growth (Zhang et al., 2022).

3.3. Evolution of microbial populations

The number and survival time of different microorganisms during fermentation play a crucial role in determining the aroma composition of fruit wines (Zhang et al., 2018). Fig. 3 illustrates the dynamics of *S. cerevisiae* and L. *plantarum* populations in various apple ciders. The population of *S. cerevisiae* in apple ciders reached a peak of 5.9×10^8–7.3×10^8 CFU/mL on day 2 and subsequently declined. When L. *plantarum* was added to the mixed fermentation treatments, the population of *S. cerevisiae* decreased more significantly on day 2 compared to the single-culture fermentation group. This decline may be attributed to L. *plantarum* consuming nutrients required by *S. cerevisiae* or producing yeast growth inhibitors, such as certain organic acids (Hu, Liu, Wang, & Zhang, 2020). Additionally, apart from L. *plantarum* SCFF19 and SCFF185, other L. *plantarum* strains experienced a decrease in cell counts during the late fermentation stage of apple ciders. However, the cell counts of the remaining strains increased from day 2 to day 5 of fermentation and then remained relatively stable, indicating the ability of most L. *plantarum* strains to adapt well to the cider fermentation environment, consistent with our previous findings (Chen et al., 2023).

3.4. Comparative analysis of antioxidant activity

In the present study, a comparison was made among different fermentation treatments in terms of total phenolic content, DPPH radical scavenging rate, and total antioxidant capacity. Fig. 4 demonstrates a significant increase in antioxidant activity following fermentation. Moreover, the mixed microbial fermentation groups (LAM) exhibited higher antioxidant activities when compared to the single fermentation group (ASY), which aligns with previous research findings (Wang et al., 2022).

The total phenol content serves as a vital parameter for assessing the quality of fruit wines, as it directly impacts their taste and color (He et al., 2022). Additionally, the presence of phenols in ciders offers antioxidant properties and a potential reduction in the risk of chronic diseases (Bortolini et al., 2020). Fig. 4a illustrates the oxidation, polymerization, or precipitation reactions of phenols by microorganisms during fermentation, resulting in significant overall changes in the total phenol content across different fermentation treatments (Way et al., 2019). Notably, there were significant variations in the total phenol content among the different ciders at the end of fermentation, with the mixed fermentation treatments (LAM) displaying notably higher total phenol content compared to the single fermentation group (ASY). In particular, the cider produced through co-inoculation with LAM134 exhibited the highest total phenol content at 897.57 mg/L. Hence, MLF contributed to the preservation or even enhancement of phenol content to a certain extent, consequently improving the overall quality of ciders.

As depicted in Fig. 4b, the co-fermentation treatments with *S. cerevisiae* and L. *plantarum* resulted in higher DPPH free radical scavenging rates in cider samples compared to those in apple juice (AJ) and cider from single-culture fermentation (ASY). The DPPH free radical scavenging rate in co-fermented ciders reached its peak on day 14 after the addition of L. *plantarum*, indicating an improved utilization of polyphenolic compounds by L. *plantarum* (Santos Filho et al., 2019). Furthermore, the DPPH radical scavenging rates of ciders from different fermentation treatments with various L. *plantarum* strains exhibited significant differences. Remarkably, the treatment with LAM284 showed the highest DPPH radical scavenging rate at 85.34%, highlighting the substantial enhancement of the DPPH radical scavenging rate in ciders through MLF with the addition of L. *plantarum*.

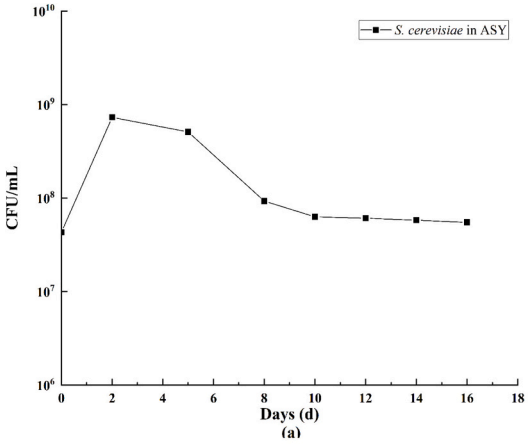
Fig. 4c depicts the dynamic changes in the total antioxidant capacity in all fermentation samples. The total antioxidant capacity of ciders exhibited a significant increase on day 2 and reached its maximum on the 12th day, with the treatment involving LAM284 displaying the highest antioxidant capacity at 365.60 mmol/L. Overall, the total antioxidant capacity of ciders in the mixed microbial fermentation treatment (LAM) surpassed that of both apple juice (AJ) (112.79 mmol/L) and the single fermentation group (ASY) (220.92 mmol/L). These findings indicate that the addition of L. *plantarum* during simultaneous AF and MLF enhanced the total antioxidant capacity of ciders, aligning with previous research (Kwaw et al., 2018).

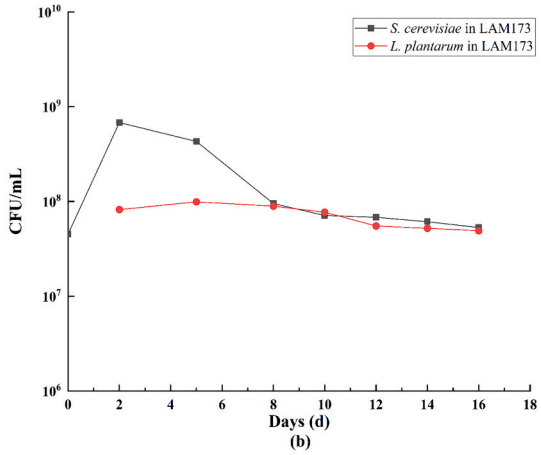
3.5. Comparative analysis of aroma composition

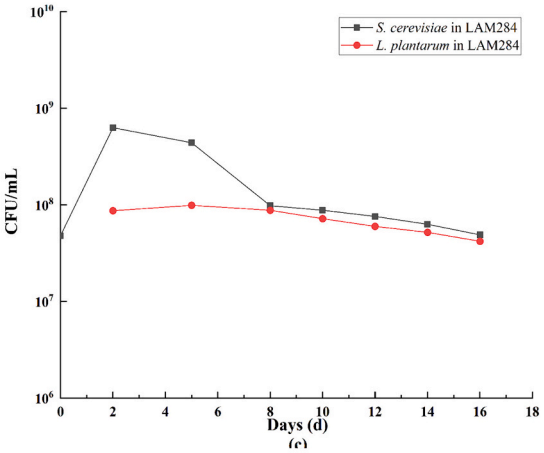
Volatile compounds play crucial roles in determining the consumer palatability of fruit wines (Kaprasob et al., 2017). Previous studies on fruit wines have demonstrated the significant contribution of LAB to the production of volatile substances during fermentation (Knoll et al., 2011). In this study, a comprehensive analysis (Table S1) detected 72 volatile compounds in all samples, including esters, higher alcohols, fatty acids, aldehydes, and other volatile compounds. Notably, the total volatile substance content in ciders from mixed fermentations with *S. cerevisiae* and L. *plantarum* exhibited a significant increase compared to the single-culture fermentation cider (ASY). Among the treatments, the LAM180 treatment displayed the highest volatile compound content at 20760.42 ± 211.26 μg/L. These findings suggested that simultaneous AF and MLF with the addition of L. *plantarum* enhanced the production of aroma compounds, supporting earlier research that the co-fermentation of *S. cerevisiae* and *L. plantarum* contributed to optimizing the overall aroma profile and complexity of the ciders (Peng et al., 2021).

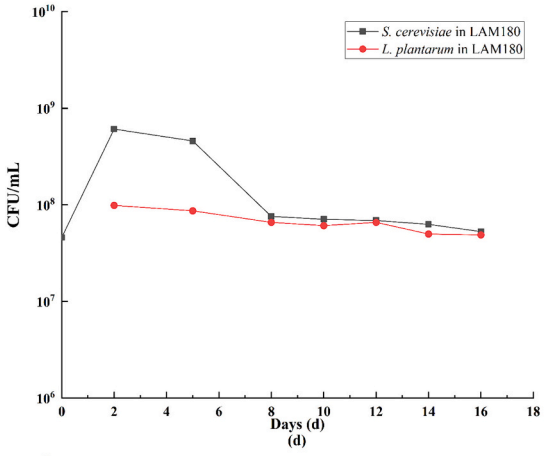
Based on the analysis of the cluster heatmap presented in Fig. 5a, noticeable variations in the volatile compound composition were observed between apple juice and apple ciders. The color blue in the heatmap represents up-regulated compounds, while the color orange represents down-regulated compounds after fermentation. The samples from apple juice and ciders were categorized into three clusters. Specifically, the samples from apple juice and the cider fermented with a single culture of *S. cerevisiae* were grouped into clusters W1 and W2, respectively. Conversely, the samples from apple ciders fermented with both *S. cerevisiae* and *L. plantarum* were grouped into cluster W3, which exhibited higher levels of aroma compounds like Hexyl 3-methyl butanoate and 1-Deoxy-D-mannitol. Furthermore, ciders fermented with *S. cerevisiae* and different strains of L. *plantarum* were individually grouped, suggesting that the aroma composition in fermented ciders highly depends on the specific strain used. This finding further supports earlier research (Wang et al., 2022), indicating that the addition of L. *plantarum* during cider fermentation enhances the production of volatile compounds and contributes to the complexity of its aroma.

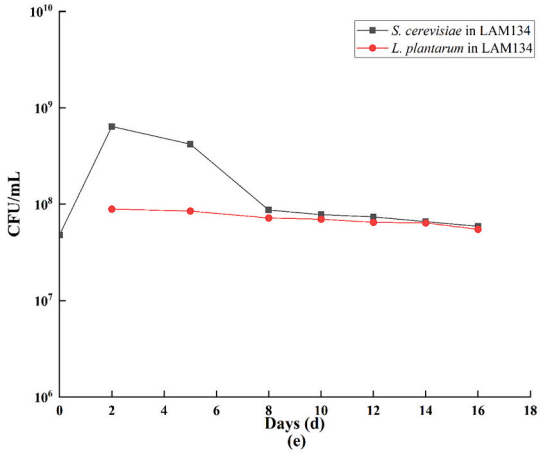
PCA was utilized to further examine the patterns and distinctions in the volatile profiles of apple juice, and ciders fermented with single-culture and co-cultures (Fig. 5b). PC1 accounted for 31.3% of the total variance, while PC2 accounted for 20.6%. As depicted in Fig. 5b, the PCA scatter plot demonstrated the differentiation between apple juice and ciders fermented with single-culture and co-cultures. The fermented cider treatments were located in different quadrants. Particularly, the samples from the LAM19 and LAM134 treatments were grouped in the first quadrant, displaying positive values of PC1 and PC2, and exhibited strong correlations with ethyl acetate, ethyl tetradecanoate, 1-butanol, and isoamyl acetate content. In contrast, the samples from the LAM185 treatment were positioned in the second quadrant, revealing correlations with 2-methyl n-butanol and 4-ethyl benzaldehyde. Apple juice (AJ) and the single culture of cider (ASY) were in the third quadrant, characterized by negative values of PC1 and PC2, and were associated with caproaldehyde and butyl acetate content. Lastly, the samples from the LAM180, LAM284, and LAM173 treatments were found in the fourth quadrant, displaying positive PC1 values and negative PC2 values, and were predominantly correlated with phenethyl acetate, ethyl dodecanoate, and acetic acid content. These different treatments presented inconsistent correlations with different compounds may be due to cell-cell interaction and nutritional competition between *S. cerevisiae* and different L. *plantarum* strains (Chen et al., 2022; Hu

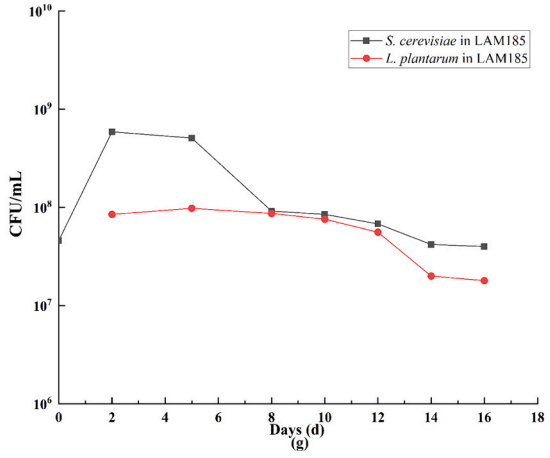












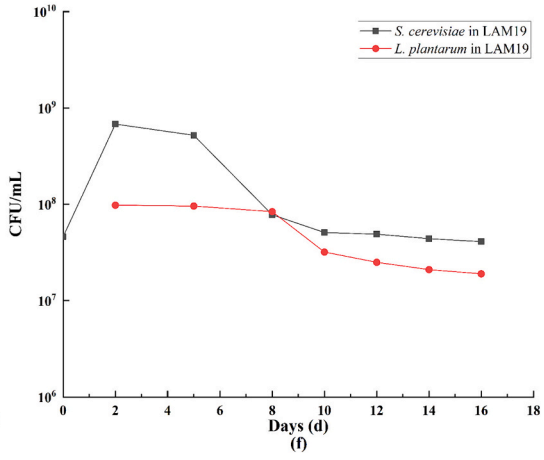


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Fig. 3. Dynamic changes of the total cell counts of *S. cerevisiae* and L. *plantarum* in apple ciders. ASY: apple cider inoculated with *S. cerevisiae* SY, LAM173: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF173, LAM284: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF284, LAM180: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF180, LAM134: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF134, LAM19: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF19, LAM185: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF185.

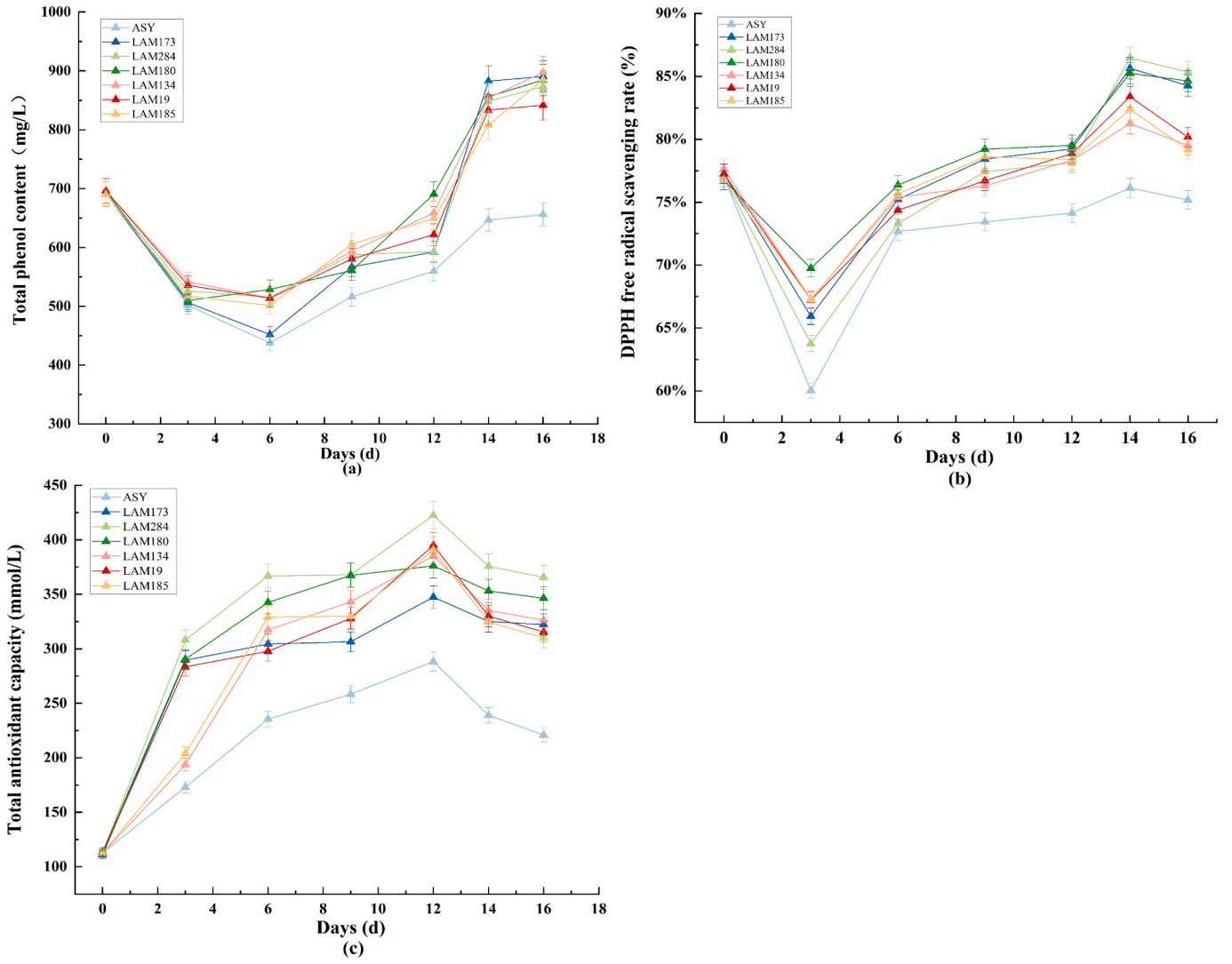
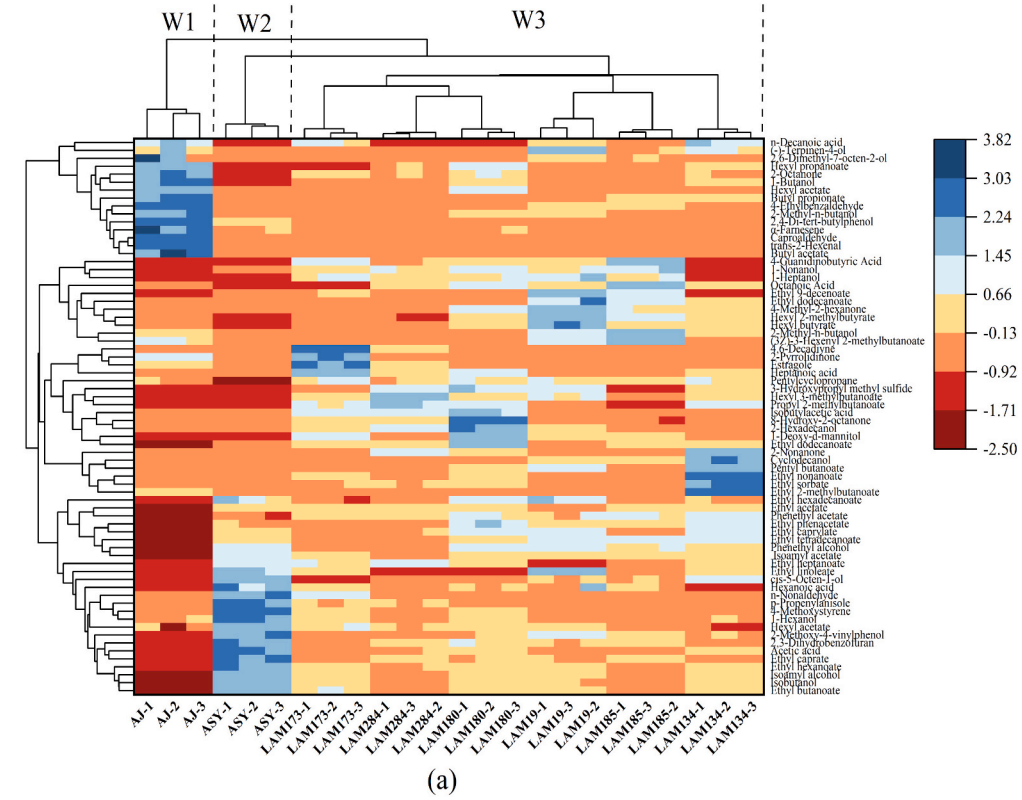


Fig. 4. Dynamic evolution of the total phenols content (a), DPPH free radical scavenging rate (b), and total antioxidant capacity (c) in pure and mixed culture fermentations. ASY: apple cider inoculated with *S. cerevisiae* SY, LAM173: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF173, LAM284: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF284, LAM180: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF180, LAM134: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF134, LAM19: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF19, LAM185: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF185.

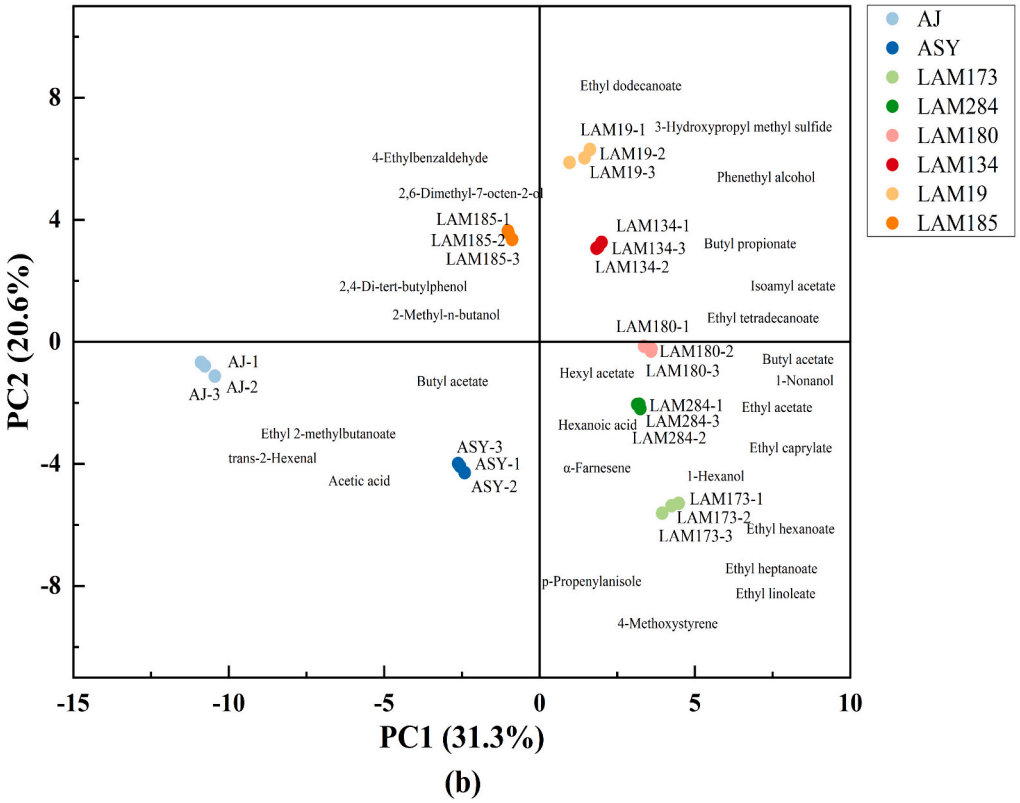
et al., 2022).

Esters emerged as the most abundant volatile compounds in apple juice and ciders, as outlined in Table S1. These compounds are vital in shaping key cider characteristics, particularly the desired fruity aromas (Cai et al., 2020). The ester content in apple juice (AJ) was found to be merely 354.27 ± 12.46 μg/L. However, after fermentation, a significant increase in ester content was observed, with cider samples ranging from 7620.34 ± 31.01 μg/L (ASY) to 15,949.69 ± 11.30 μg/L (LAM134). Furthermore, the mixed-culture fermentation treatments (LAM) exhibited considerably higher ester contents compared to the single fermentation group (ASY), and the types and quantities of esters in ciders fermented with different L. *plantarum* strains demonstrated significant variations. Referencing Table S1, ethyl esters emerged as the predominant esters in the final cider products, with ethyl acetate, isoamyl acetate, ethyl caprylate, ethyl phenacetate, ethyl tetradecanoate, and ethyl dodecanoate standing out as the most abundant ones, imparting fruity and sweet aromas to the ciders. Consequently, the addition of L. *plantarum* during simultaneous AF and MLF appears to contribute to the accumulation of esters, aligning with earlier research findings (Zhang et al., 2022).

Higher alcohols are typically recognized for their ability to impart floral aromas to fruit wines and, when present in quantities under 300 mg/L, are considered to positively contribute to the overall quality of the wine (González Álvarez et al., 2011). As indicated in Table S1, the levels of higher alcohols in all fermented cider samples ranged from 1963.68 ± 374.24 μg/L to 4146.41 ± 41.18 μg/L, significantly surpassing that of apple juice (123.92 ± 9.17 μg/L). The prevailing higher alcohols found in apple ciders were isoamyl alcohol, phenyl ethanol,



(a)



(b)

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Fig. 5. Volatile compounds in apple juice and ciders produced by single-culture and mixed-culture fermentations. Cluster heatmap of volatile compounds (a) in apple juice and ciders. Principal component analysis (PCA) (b) on the basis of the volatile substances of apple juice and ciders. AJ: apple juice without inoculation, ASY: apple cider inoculated with *S. cerevisiae* SY, LAM173: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF173, LAM284: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF284, LAM180: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF180, LAM134: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF134, LAM19: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF19, LAM185: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF185.

isobutanol, and n-hexanol, which aligns with the findings of a previous study (Peng et al., 2015). Specifically, the LAM173 treatment exhibited concentrations of 1907.43 ± 53.51 μg/L and 143.99 ± 6.20 μg/L for isoamyl alcohol and isobutanol, respectively, which contributed fruity and whiskey-like aromas to the ciders (Li, Bi, et al., 2022; Li, Zhang, et al., 2022). Furthermore, the LAM180 treatment showcased the highest levels of phenylethyl alcohol (212.71 ± 12.12 μg/L), known for providing floral and rose scents (Selli et al., 2008). These findings suggested that the addition of L. *plantarum* during simultaneous AF and MLF played a significant role in augmenting the content of higher alcohols, thereby enhancing the aroma of the ciders.

In addition, the levels of fatty acids, aldehydes, and other aroma compounds were determined in both the apple juice and the final cider samples. Volatile fatty acids contribute to the complexity of cider aromas by imparting a sweet and acidic profile, although concentrations exceeding 20 mg/L can result in a rancid taste in cider (He et al., 2021). The data presented in Table S1 showed higher levels of volatile fatty acids in the final ciders (ranging from 195.85 ± 13.50 μg/L to 562.06 ± 33.06 μg/L) compared to the initial apple juice (5.64 ± 1.24 μg/L). It is commonly understood that the presence of aldehydes in apple ciders contributes to off-flavors (Zhong et al., 2021). Interestingly, this study revealed that ciders fermented with a combination of *S. cerevisiae* and L. *plantarum* exhibited significantly lower levels of aldehydes compared to those fermented with a single culture. Consequently, the addition of *L. plantarum* strains during simultaneous AF and MLF facilitated the enrichment of esters, higher alcohols, and fatty acids, while simultaneously reducing the presence of aldehydes in the ciders.

3.6. Sensory evaluation

The sensory evaluation results of the cider samples are presented in Fig. 6. Compared to ciders fermented solely with *S. cerevisiae*, the ciders fermented with *S. cerevisiae* and *L. plantarum* gained significantly higher sensory scores. Moreover, the floral, fruity, sweet, and overall acceptability attributes of the co-culture fermented ciders were notably enhanced, particularly in the case of LAM134 cider, suggesting that the addition of *L. plantarum* during simultaneous AF and MLF positively influenced cider acidity, consistent with prior studies (Zhang et al., 2022). Furthermore, the sensory evaluation findings revealed notable discrepancies among the apple ciders fermented with *S. cerevisiae* and different strains of L. *plantarum*, underscoring the significance of strain-level differences in L. *plantarum* co-fermentation as a pivotal factor affecting the sensory qualities of the ciders, which aligns with previous research findings (Li, Zhu, & Zhao, 2020).

4. Conclusions

The chemical composition and desired properties of cider play a crucial role in selecting suitable LAB strains for MLF, as they directly impact the final quality of ciders. In this study, it was observed that the presence of L. *plantarum* had a significant influence on shaping the cider's quality during mixed fermentations. Our findings underscored the potential efficacy of six specific strains of L. *plantarum* as MLF starters. This specificity was attributed to their distinctive enological properties, resulting in variations in basic parameters, antioxidant activity, MLF performance, and aroma profiles of the final ciders. Notably, in addition to enhancing the contents of malic acid and lactic acid contents, each L.

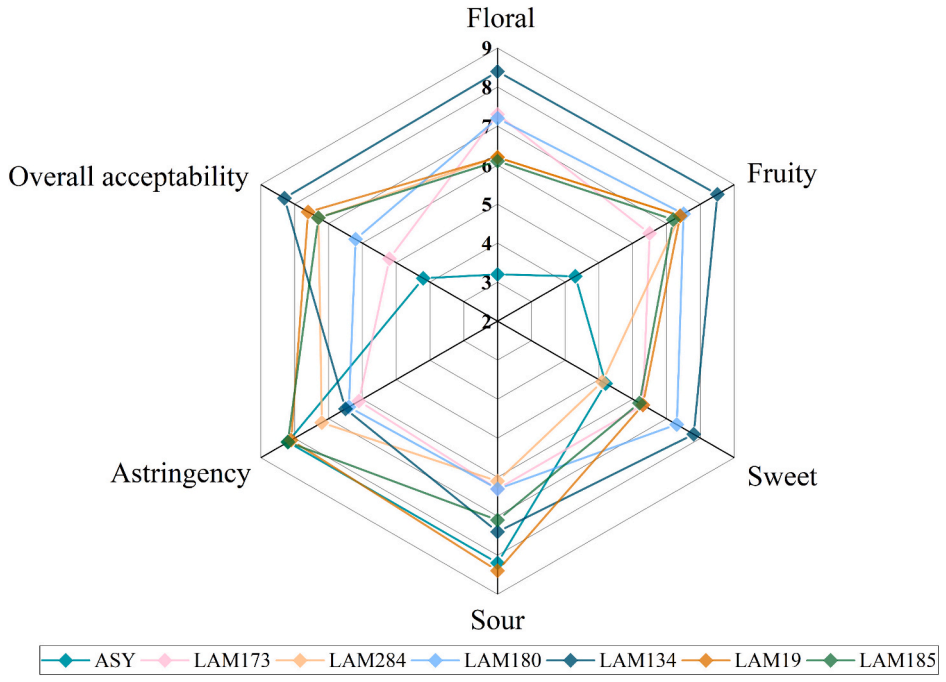

Fig. 6. Sensory profiles obtained for ciders produced by single-culture and mixed-culture fermentations. ASY: apple cider inoculated with *S. cerevisiae* SY, LAM173: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF173, LAM284: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF284, LAM180: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF180, LAM134: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF134, LAM19: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF19, LAM185: apple cider co-inoculated with *S. cerevisiae* SY and *L. plantarum* SCFF185.

plantarum strain exhibited a unique and different impact on cider antioxidant activity and modulation of sensory profiles. Consequently, this study confirmed the viability of utilizing L. *plantarum* strains as an alternative approach to traditional cider production through MLF induction. Furthermore, when selecting L. *plantarum* strains in multi-starter cider fermentations, greater attention should be paid to L. *plantarum* at the strain level. Future research endeavors should explore the outcomes of employing different apple varieties in this cider-making practice and examine the performance of L. *plantarum* strains during simultaneous AF and MLF at an industrial scale. Additionally, further investigation is warranted to gain insights into the mechanisms underlying MLF performed by L. *plantarum* in cider production.

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CRediT authorship contribution statement

Lujun Hu: Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Xiaodie Chen:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Yulan Cao:** Writing – review & editing, Validation, Supervision. **Pei Gao:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Teng Xu:** Validation, Supervision. **Dake Xiong:** Validation, Supervision. **Zhifeng Zhao:** Validation, Supervision.

Declaration of competing interest

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

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101575.

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