

Multi-ratio co-inoculation of *Lactiplantibacillus plantarum* and *Oenococcus oeni* with *Saccharomyces cerevisiae* reveals an optimal blend for enhancing aroma complexity and sensory preference of Cabernet Gernischt wine

Yaxin Cao

Ludong University

Shuyang Sun

sysun81@163.com

Ludong University

Ziruo Sun

Ludong University

Taihong Liu

Ludong University

Wenguang Jiang

Ningxia Changyu Longyu Estate Co. Ltd

Article

Keywords: Multi-ratio co-inoculation, *Lactiplantibacillus plantarum*, *Oenococcus oeni*, wine, volatile analysis

Posted Date: July 23rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10245387/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

The interaction between *Saccharomyces cerevisiae* and co-inoculated lactic acid bacteria (LAB) at defined ratios during simultaneous alcoholic and malolactic fermentation remains largely unexplored, offering a unique perspective that provides new options for winemakers and researchers. This is the first report on the investigation of five LAB inoculation strategies: single *Oenococcus oeni* (OO), single *Lactiplantibacillus plantarum* (LP), and mixed ratios of 1:1 (O1L1), 4:1 (O4L1), and 1:4 (O1L4) co-cultured with yeast. Mixed LAB accelerated fermentation, and *L. plantarum* dominated mixed cultures regardless of initial ratio. A total of 85 volatiles were identified, and each treatment displayed characteristic compounds: OO was distinguished by furfural and 2,3-butanedione; LP by ethyl nonanoate and 4-terpineol; O4L1 by octanoic acid and methyl octanoate; O1L1 by α -terpineol and ethyl butyrate; O1L4 by ethyl lactate and 2-phenylethanol. Sensory evaluation ranked O1L1 highest, with enhanced fruity and floral notes and suppressed green and herbal characters. An OAV-PLS model identified spicy and green as key discriminators. Residual analysis showed O1L1 exhibited the smallest residuals, confirming 1:1 as the optimal ratio for metabolic complementarity and aroma balance. Overall, these findings provide rational design of LAB co-inoculation with yeast for targeted wine aroma.

1. Introduction

Malolactic fermentation (MLF), a secondary fermentation process usually conducted during or after alcoholic fermentation, is characterized by the bioconversion of L-malic acid to L-lactic acid dominated by lactic acid bacteria (LAB)¹. This metabolic transformation enhances microbial stability and sensory complexity in wines. *Oenococcus oeni* and *Lactiplantibacillus plantarum* are primary starters used in MLF², attributed to their robust tolerance to low pH and high ethanol concentrations, efficient deacidification capacity and contribution to wine's complexity^{3,4}.

Previous research on the relationship between MLF starters and wine aroma has primarily focused on three aspects⁵⁻⁸: 1) the impact of combined fermentation (involving *Saccharomyces* and non-*Saccharomyces* yeasts followed by specific LAB); 2) inoculation protocols (sequential yeast-LAB inoculation versus co-inoculation); 3) comparative effects of *O. oeni* versus *L. plantarum* on wine aroma. However, studies exploring blended MLF starter cultures (concurrent use of multiple LAB strains) remain limited. *L. plantarum* and *O. oeni* have distinct metabolic activities and different flavor enzyme profiles, such as glycosidases, esterases and citrate lyases over MLF, therefore, it is reasonable to speculate that their interactions may bring great changes in the aromatic and sensory profiles of wine products.

Devi and Anu-Appaiah evaluated the compatibility of mixed-strain MLF using *L. plantarum* and *O. oeni* with different *S. cerevisiae* strains and their impact on the phenolic and volatile constituents of Shiraz wines⁹. They found that this approach led to evident improvement in the chemical and sensory profile compared to single-strain MLF. Subsequently, the same research group investigated the timing of inoculation for these mixed MLF starters during Shiraz vinification, revealing that the resulting wines received superior sensory scores for color, body, and overall acceptability compared to traditionally late-inoculated wines¹⁰. Similarly, Zhang et al. examined the co-culture of indigenous *O. oeni* and *L. plantarum* for driving MLF in *Cabernet Sauvignon* and its interactive impact on wine quality. And they found that the two-species culture achieved faster and more complete fermentation, significantly elevated desirable volatile compounds, and reduced color loss compared to single-strain inoculations¹¹.

These studies have substantially advanced the understanding of mixed-strain MLF and its oenological implications. However, the interaction dynamics between co-cultured LAB strains, particularly regarding specific inoculation ratios and their competitive kinetics remain inadequately characterized. Moreover, research on mixed LAB cultures conducted simultaneously with alcoholic fermentation is still limited. In such scenarios, three major microbes including *S. cerevisiae*, *L. plantarum*, and *O. oeni* coexist in the fermentation process, creating a complex microbial ecosystem. The metabolic crosstalk and competitive interactions among these three species may lead to chemical and sensory outcomes that differ from those observed in sequential fermentation or single-strain culture. Therefore, a systematic investigation into how specific LAB blend ratios influence microbial succession, volatile compound modulation, and final sensory attributes under simultaneous fermentation should be conducted.

Here, we investigate the effects of defined inoculation ratios of *L. plantarum* and *O. oeni* (OO, LP, O1L1, O1L4 and O4L1; Fig. 1). Throughout fermentation, the establishment and persistence of inoculated microbes were monitored, and after MLF the resulting wines were analyzed for enological parameters, volatile compound profiles and sensory attributes. Specifically, volatile compounds were characterized using headspace solid-phase microextraction coupled with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (HS-SPME-GC $\times$ GC-TOF-MS). This advanced analytical approach generates highly structured two-dimensional chromatograms, in which compounds are resolved based on both volatility and polarity, thereby offering enhanced peak capacity and sensitivity for profiling complex wine aroma matrices¹². This study aims to elucidate how specific LAB co-inoculation ratios modulate wine quality under concurrent fermentation conditions, thereby offering winemakers a practical strategy to produce wines with more desirable sensory profiles.

2. Results and Discussion

2.1 Fermentation kinetics

2.1.1 Alcoholic fermentation and yeast viability

In this study, the growth kinetics of *S. cerevisiae* and the consumption of reducing sugars during AF were quantitatively determined in response to different LAB and inoculation ratio. In the single MLF strain treatments, yeast in the LP fermentation peaked at 1.17×10^8 CFU/mL on the 3rd day; in contrast, yeast in the OO treatment reached a lower maximum of around 1.02×10^8 CFU/mL at the same time. Among the co-culture treatments, the growth patterns varied with the inoculation ratio. The O1L4 treatment promoted a vigorous yeast growth, achieving the highest peak density of 1.82×10^8 CFU/mL by day 3 and maintaining a higher cell count thereafter. The yeast growth in the O1L1 treatment achieved its maximum of 1.41×10^8 CFU/mL also on the 3rd day. A different pattern was notable in the O4L1 treatment, where the yeast population reached an earlier peak of 1.23×10^8 CFU/mL on the 2nd day, followed by a more marked decline (Fig. 2a).

As seen in Fig. 2b, the degradation patterns of reducing sugars corresponded with the observed yeast growth profiles. All treatments showed a consistent decline in sugar concentration from initial levels. The single-strain OO treatment exhibited the slowest consumption rate, retaining 10.3 g/L on 6th day and 1.8 g/L on 7th day. The LP treatment, associated with stronger yeast growth, demonstrated more efficient sugar utilization, depleting sugars to 1.5 g/L by day 6. The co-culture treatments generally displayed accelerated sugar consumption. The O1L4 treatment was the most effective, reducing sugar to 3.3 g/L on 6th day. The O1L1 and O4L1 treatments also showed enhanced consumption compared to the OO control, reaching 2.8 g/L and 7.3 g/L by day 6, and 2.1 g/L by day 7 for O4L1, respectively.

These results indicate that the presence and ratio of *L. plantarum* in co-culture with *O. oeni* insignificantly affected the alcoholic fermentation kinetics. A higher proportion of *L. plantarum* (O1L4) seemed to be associated with both enhanced peak yeast biomass and more complete sugar consumption, suggesting a synergistic or favorable effect on the fermentation environment for *S. cerevisiae*. In contrast, a dominance of *O. oeni* (O4L1) correlated with an earlier but lower yeast peak and a comparatively slower rate of sugar degradation.

2.1.2 Malolactic fermentation and LAB viability

2.1.2.1 LAB viability

The growth of LAB and the progression of MLF were also monitored under different inoculation modes. Bacterial community dynamics were monitored at the early (day 3), mid (day 8 for all wines except OO and day 9 for OO), and late (day 12 for all samples except OO and day 14 for OO) fermentation stages following yeast inoculation (Fig. 3a). At the early stage, OTU numbers varied considerably across treatments, ranging from 313 to 553. The highest diversity was observed in the *O. oeni* monoculture, while the O1L1 co-culture group exhibited relatively lower OTU numbers. By the mid-fermentation stage, the total number of OTUs decreased to a range of 358~438 across treatments, indicating a progressive dominance of bacterial populations better adapted to the wine environment. This trend continued into the late stage, where OTU counts further declined to 327~397, reflecting the near-complete establishment of the inoculated LAB and the effective suppression of the indigenous microbiota.

Fig. 3b illustrated the progression of bacterial genera across five treatments. By day 3, community composition was influenced by the inoculation strategy. LP was dominated by *Lactobacillus* (62.4%), and retained a substantial reservoir of non-LAB populations (34.6%), including *Acetobacter*, *Bacillus* and *Acinetobacter*. OO was dominated by *Oenococcus* (58.2%), with relatively larger proportions of *Leuconostoc* (1.9%), *Kluyvera* (1.2%) and *Lactobacillus* (5.3%). Mixed LAB treatments displayed ratio-dependent outcomes: O1L4 showed dominance of *Lactobacillus* (63.5%), followed by *Oenococcus* (8.6%), with residual non-LAB genera such as *Sphingomonas* and *Rhodococcus* together comprising 27.8%; O1L1 revealed near-equilibrium competition between *Oenococcus* (44.7%) and *Lactobacillus* (43.9%), while maintaining the highest non-LAB diversity (11.4%); and O4L1 presented *Oenococcus* at 58.3% and *Lactobacillus* at 22.1%.

By mid-fermentation, community structures underwent marked changes. In O1L4, *Lactobacillus* proliferated markedly to 77.3%, while *Oenococcus* declined to 6.9%. O1L1 showed a similar but moderated trend, with *Lactobacillus* reaching 68.5% and *Oenococcus* 18.4%, indicative of the competitive superiority of *L. plantarum* at this inoculation ratio. Notably, O4L1 maintained an equilibrium between *Lactobacillus* (44.1%) and *Oenococcus* (46.3%). This suggests that despite a high initial *O. oeni* density, *L. plantarum* was able to effectively compete, preventing *O. oeni* from prosperous growth. Across all mixed LAB cultures, indigenous non-LAB populations were uniformly suppressed to a collective abundance of 10~13%. Meanwhile, the single LAB cultures achieved near-complete dominance by the inoculated strain, as seen by *Lactobacillus* covering 87.2% in LP and *Oenococcus* comprising 75.1% in OO.

At the late fermentation stage, the bacterial community reached terminal equilibrium states. O1L4 showed the prevalence of *Lactobacillus* (82.4%) and lower abundance of *Oenococcus* (9.2%). O1L1 exhibited a similar trend, with *Lactobacillus* dominating at 88.2% and *Oenococcus* at 11.8%. In contrast, O4L1 maintained substantial populations of both *Lactobacillus* (53.5%) and *Oenococcus* (38.6%). And non-LAB genera were nearly eliminated across all mixed LAB treatments, jointly accounting for 5.7~8.4% of the community.

The progression of microbial diversity reveals a clear distinction between single and mixed LAB inoculations in affecting bacterial communities during concurrent AF and MLF. LP and OO exhibited limited LAB diversity but still retained detectable non-LAB populations. In contrast, mixed LAB inoculations supported coexistence of LAB species throughout fermentation, although competitive outcomes consistently favored *L. plantarum*. And the reason can be attributed to three aspects. First, *L. plantarum* possesses broader metabolic versatility and utilizes a more diverse range of carbon sources compared to *O. oeni*^{13, 14}, enabling it to proliferate as fermentation progresses. Second, under moderate acidic conditions (pH >

3.5) in this study, *L. plantarum* exhibits relative higher tolerance to wine-related stresses, through certain quorum sensing system and critical component biosynthesis pathway, which help maintain metabolic fitness under harsh conditions^{15,16}. Third, *L. plantarum* exhibits an active antagonistic effect through the production of antimicrobial compounds¹⁷, particularly bacteriocins, and these bacteriocins exert potent inhibitory effects against other LAB including *O. oeni*, thereby suppressing competitor populations and securing ecological dominance in mixed fermentation environments.

2.1.2.2 Malic acid metabolism

Alongside the analyses of bacterial communities, the reductions of malic acid during MLF were determined. As seen in Fig. 4, evident differences in malic acid metabolism were observed between the two single-strain inoculations. MLF driven solely by *L. plantarum* exhibited a more rapid degradation rate, with malic acid concentration decreasing below 0.3 g/L over a 13 day fermentation period, indicating a highly efficient and complete MLF process. In contrast, fermentation with *O. oeni* proceeded at a much slower rate, retaining approximately 0.88 g/L malic acid at day 14 and fermentation was completed by day 17. Compared to OO, all mixed LAB cultures exhibited significantly accelerated malic acid consumption. From day 3 onward, malic acid levels in the mixed cultures were consistently lower than those in *O. oeni* mono-culture. Furthermore, different kinetic profiles emerged among the mixed LAB treatments depending on the inoculation ratio: O1L4 exhibited the most rapid degradation throughout fermentation, achieving complete MLF within 14 days; O1L1 displayed an intermediate degradation rate, and by day 11 it had reduced malic acid below 1 g/L; O4L1, initially dominated by *O. oeni*, showed the slowest degradation kinetics among the mixed cultures during early and mid-fermentation, but an acceleration occurred after day 9 and MLF was also completed by day 15.

2.2 Basic composition

The basic physicochemical parameters of the wines following the completion of fermentation are summarized in Table 1. Residual reducing sugar concentrations ranged from 0.9 to 3.2 g/L across treatments. Alcohol content varied between 11.5% and 12.1% (v/v). Total acidity ranged from 4.95 to 5.13 g/L, with no statistically significant differences observed among treatments. Similarly, volatile acidity remained within acceptable limits across all samples (0.36–0.46 g/L), considerably below the sensory threshold of 1.2 g/L, confirming that no undesirable microbial activity occurred during fermentation. The pH values showed minimal variation, ranging from 3.62 to 3.68, while soluble solids content ranged from 7.95% to 8.17%, with O1L4 exhibiting the highest value. These data demonstrate that despite the distinct inoculation strategies and LAB species employed, all treatments produced wines with comparable and commercially acceptable chemical compositions, confirming the suitability of mixed LAB co-inoculation for practical winemaking applications.

2.3 Volatile profiles and principal component analysis

2.3.1 Comparison of volatile compounds

To characterize the volatile profiles of wines derived from different LAB inoculation strategies, volatile compounds were analyzed using HS-SPME coupled with GCxGC-MS. A total of 79 volatile compounds were identified and quantified across all samples, including 28 esters, 22 alcohols, 7 acids, 9 aldehydes, 6 ketones, and 7 terpenes. The concentrations of volatile compounds are provided in Fig. 5 and Supplementary material (Table S1).

Esters constituted the most distinctive group of volatile compounds in the wines, and ethyl acetate, ethyl butyrate, ethyl hexanoate and ethyl decanoate are major representatives. OO was characterized by highest concentrations of ethyl propanoate, ethyl heptanoate, methyl nonanoate and methyl salicylate; while LP produced significantly higher levels of ethyl acetate, ethyl nonanoate, isoamyl octanoate, diethyl succinate, ethyl 2-phenylacetate and ethyl 3-methylbutanoate. In mixed LAB cultures, synergistic interactions between *L. plantarum* and *O. oeni* led to a notable enhancement of several important esters. O1L1 exhibited evident enhancement of many esters including ethyl propanoate, ethyl butyrate, ethyl hexanoate, ethyl 3-methylbutanoate, isoamyl lactate, ethyl decanoate, diethyl succinate, ethyl 9-decanoate, ethyl undecanoate and 2-phenylethyl hexanoate. Notably, the concentration of ethyl butyrate in O1L1 was 1.25-fold higher than in OO and 1.13-fold greater than in LP. Similarly, ethyl hexanoate levels in O1L1 reached 1.6-fold that of O1L4, while isoamyl lactate showed a marked increase of about 1.37-fold compared to LP. O4L1 favored the production of methyl octanoate, ethyl octanoate, ethyl 9-decanoate, ethyl dodecanoate and 2-phenylethyl hexanoate. For example, the concentration of methyl octanoate in O4L1 reached 1.36-fold that in LP, while ethyl dodecanoate was about 1.3-fold higher than in OO. Moreover, O1L4 led to elevated levels of ethyl butyrate, ethyl hexanoate, ethyl lactate, ethyl 2-hydroxybutanoate, ethyl octanoate, ethyl benzoate.

Alcohols constituted another major group of volatile compounds identified in the wines. A total of 22 volatile alcohols were detected, with isoamyl alcohol, 2-phenylethanol, and 1-hexanol being the most abundant. OO was characterized by higher concentrations of propanol, isoamyl alcohol, 1-hexanol and 1-decanol, while LP showed the greatest abundance of ethanol, 2-methylbutanol, 2-hexanol and 1-pentanol. Mixed-culture MLF evidently reduced the total concentration of higher alcohols compared to single cultures, and different modulations were observed among the co-cultures. O1L1 exhibited the highest levels of (Z)-3-hexen-1-ol, 1-heptanol and 1-nonanol. O1L4 was notable for greater production of 2-methylpropanol, benzyl alcohol and 2-phenylethanol. Moreover, O4L1 showed the highest concentrations of 1-butanol, (Z)-2-hexen-1-ol, 2,3-butanediol and 1-octanol, suggesting that a higher proportion of *O. oeni* in initial mixed inoculations changed the metabolic equilibrium toward the accumulation of these compounds.

A total of 7 volatile acids were quantified among the experimental wines. OO displayed the higher concentration of decanoic acid, and LP was characterized by significantly elevated levels of acetic acid. Among the mixed-cultures, O4L1 showed the greatest abundance of hexanoic and octanoic acids, O1L1 exhibited higher concentrations of propanoic, butanoic, and nonanoic acids, while O1L4 demonstrated an intermediate profile with moderate levels of most acids. These findings were consistent with observations in Shiraz wines, where mixed LAB cultures were reported to modify acid profiles through microbial interaction ⁹.

A total of 9 aldehydes were quantified in the wine samples, with 3-methyl-butanol, hexanal and benzaldehyde as major representatives. OO showed elevated levels of hexanal, octanal and furfural, with the content of the former two compounds being 1.30 and 1.18 times greater than the average in other treatments. LP had enhanced formation of undecanal. O1L4 showed greater production of 4-ethylbenzaldehyde, and its concentration reached 1.24 and 1.17 times that in OO and O4L1, respectively. O1L1 exhibited marked accumulation of 3-methyl-butanol, decanal. Particularly notable was the content of 3-methyl-butanol, which was approximately 1.3-fold higher than in LP. O4L1 showed enhanced production of benzaldehyde, with the content being 1.56 and 1.35 times greater than in O1L1 and LP, respectively. Meanwhile, O1L4 was characterized by the abundance of 4-ethylbenzaldehyde and phenylacetaldehyde.

A total of 4 volatile ketones were identified in the wine samples, including 2,3-butanedione, 2,3-pentanedione, 3-hydroxy-2-butanone and 2-nonanone. O1L1 was characterized by the highest concentrations of 3-hydroxy-2-butanone and 2,3-pentanedione, with the level of the former component being 1.35 and 1.52 times greater than those in LP and O4L1, respectively. OO exhibited the most abundant 2,3-butanedione, reaching a concentration about 1.89 times higher than in O4L1. O4L1 was distinguished by its elevated production of 2-nonanone, which was around 1.52-fold greater than in O1L1. LP and O1L4 generally presented moderated ketone amounts of most ketones.

A total of 7 terpenes were identified in the tested wines, mainly involving limonene, geraniol and 4-terpineol. OO had the highest accumulation of limonene, with its concentration being 1.38 and 1.53-fold higher than in LP and O4L1, respectively. LP was characterized by elevated production of linalool, citronellol and 4-terpineol, where 4-terpineol content was 1.51 and 1.43 times greater than in OO and O1L1, respectively. O1L1 was distinguished by higher levels of geraniol, α -terpineol and β -damascenone, with the geraniol content being 1.25 and 1.19 times greater than in LP and O1L4, respectively. And O4L1 and O1L4 had moderate concentrations of most terpenes.

2.3.2 Principal component analysis

To elucidate the compositional differences among wines subjected to different treatments, principal component analysis (PCA) was performed (Fig. 5C and D). The generated data set explained 71.84% of the total variance, with PC1 and PC2 accounted for 53.17% and 18.66%, respectively. LP was positioned on the negative side of PC1, primarily driven by elevated concentrations of ethyl acetate, isobutanol, isoamyl alcohol, 4-terpineol and acetic acid. OO was located on the positive side of PC1 near the PC2 axis, associated with higher levels of propanol, isoamyl acetate and 1-butanol. O4L1 was situated on the positive side of PC1 toward the positive PC2 direction, showing strong associations with ethyl 2-hexenoate, 2-ethyl-1-hexanol, 1-octanol and ethyl dodecanoate. O1L1 was positioned on the positive side of PC1 toward the negative PC2 direction, characterized by elevated levels of ethyl hexanoate, decanal, 3-hydroxy-2-butanone and α -terpineol. O1L4 was located near the origin, suggesting an intermediate volatile profile between the single-strain treatments.

The observed differences across all volatile categories can be attributed to the coordinated and competitive interactions between *L. plantarum* and *O. oeni* within an environment pre-established by *S. cerevisiae*. Mixed LAB cultures have a broader enzymatic profile than single species, and their co-presence creates a synergistic effect that enables access to a wider range of substrates, leading to more complex volatile profiles. This synergy is well evidenced by the superior ester profile of O1L1. *S. cerevisiae* generates ester precursors such as amino acids and fatty acids¹⁸ and these precursors are subsequently utilized by LAB through their esterases and glycosidases, with *O. oeni* converting specific precursors while *L. plantarum* enhances esterification ⁹. Resource competition for key intermediates such as acetyl-CoA may redirect metabolic fluxes, promoting certain esters while suppressing others¹⁹, which explains why ethyl acetate decreased in all mixed cultures.

Similarly, the ratio-dependent alcohol, acid, aldehyde and terpene profiles all arise from the same basic mechanism. *S. cerevisiae* supplies a common pool of metabolic precursors involving amino acids, fatty acids, α -keto acids and sugar derivatives²⁰, and the two LAB species then compete for and cooperate in utilizing these resources through their specific enzymatic activities. For alcohols, the expression of bacterial oxidoreductases can redirect fluxes away from higher alcohol formation^{21, 22}. For acids, the LAB proportion critically directs carbon flux through pathways such as fatty acid β -oxidation, citrate metabolism and amino acid degradation²³. An example is the initial dominance of *O. oeni* in O4L1, which promotes release of hexanoic and octanoic acids via enhanced esterase-mediated hydrolysis of their ethyl esters, while the elevated propanoic and butyric acids in O1L1 may arise from synergistic metabolism of amino acids or sugar derivatives ¹. Aldehyde differences reflect differential activities such as the aldehyde reductase of *L. plantarum* (reducing hexanal and octanal) and its phenolic acid decarboxylases (enhancing 4-ethylbenzaldehyde and undecanal in LP and O1L4)²⁴. Finally, terpene profiles are primarily driven by strain-dependent glycosidase activities, with *L. plantarum* possessing a relatively broader spectrum and O1L1 benefiting from complementary activities from both bacteria, leading to more comprehensive terpene liberation^{25,26}.

2.4 OAVs of volatile compounds in wines

The odor activity values (OAV) were calculated to evaluate the sensory implications of each LAB inoculation strategy, and the results are presented in Table 2. O1L1 exhibited a relatively balanced and enhanced aromatic profile, and pairwise fold-change comparisons (Fig. 6 and Fig.S1) were made to further clarify how O1L1 differed from the other treatments.

When compared to the two single LAB inoculations, O1L1 imparted the most intense black fruit and red fruit notes while suppressing herbal and green characters. Against OO, O1L1 released significantly higher OAVs of ethyl butyrate and ethyl hexanoate, both of which contribute strong strawberry, apple, banana and flowery-fruity aromas that directly enhance red fruit perception. O1L1 also elevated β -damascenone, a potent norisoprenoid that imparts apple, rose and honey notes, thereby boosting floral and sweet fruit characters. Relative to LP, O1L1 exhibited superior OAVs for ethyl butyrate, ethyl hexanoate, geraniol and decanal. Geraniol imparts citrus and geranium floral notes, enriching the floral dimension. LP maintained higher OAVs for ethyl 3-methylbutanoate (banana, sweet fruit) and linalool (floral, muscat), which contribute to its moderate red fruit and floral perception.

When compared to the other mixed inoculations, O1L1 again achieved a more harmonious sensory outcome. O4L1 exhibited the highest OAVs for ethyl 2-hexenoate, hexanoic acid and octanoic acid. These compounds contribute spicy and cheesy off-notes. O1L1 exceeded O4L1 in OAVs for ethyl butyrate (1.38-fold), ethyl decanoate (1.26-fold), ethyl 3-methylbutanoate (1.23-fold) and 2-phenylethanol (1.28-fold), thereby imparting much stronger fruity, floral and honey-like aromas while suppressing the cheesy and spicy notes. Relative to O1L4, O1L1 presented higher OAVs of ethyl butyrate and β -damascenone, which enhance strawberry, apple, rose and honey characters. While O1L4 excelled in ethyl hexanoate and 2-phenylethanol, two compounds that impart intense flowery, rose and honey notes, and thereby may enhance its floral intensity.

Pairwise fold-change comparisons among the other treatment combinations such as OO vs LP, and O4L1 vs OO, are provided in Fig. S1. These comparisons further demonstrate the ratio-dependent modulation of volatile profiles, confirming that varying the initial proportion of *O. oeni* to *L. plantarum* in a microbial ecosystem in the presence of *S. cerevisiae* systematically shifts the aromatic outcome.

2.5 Sensory analysis and PLS validation

2.5.1 Sensory evaluation

The sensory profiles of the tested wines were evaluated by a trained panel using quantitative descriptive analysis. As seen in Fig. 7A, OO exhibited medium intensity in black fruit, red fruit and floral notes, with relatively noticeable woody and green characteristics. LP showed more intense red fruit and floral notes than OO, along with moderate woody and green attributes, but its black fruit characteristic was relatively weak. O4L1 was distinguished by higher floral and spice scores, accompanied by moderate black fruit, low red fruit and green notes, and the weakest woody intensity across the samples. O1L1 was rated with the highest black fruit and red fruit ratings and the lowest green score. O1L4 achieved the highest floral intensity, and also showed higher black fruit and red fruit notes, while its woody and green attributes were relatively weak. Overall preference was highest for O1L1, followed by O1L4 and O4L1, while OO and LP received relatively lower scores.

3.5.2 OAV-based prediction of aroma dimensions and residual analysis

To investigate the regulatory effects of different *O. oeni*/*L. plantarum* inoculation ratios on wine aroma profiles, the 32 odor active compounds identified by GC $\times$ GC-MS were clustered into six OAV aroma dimensions, including black fruit, woody, spicy, green, red fruit and floral, according to their odour descriptions. A partial least squares regression (PLS-R) analysis was employed to establish a quantitative predictive relationship between the OAV dimensions and the sensory scores (Fig. 7B), and the predictive stability of the model was evaluated by leave-one-out cross-validation (LOOCV). Results showed that the model had good overall predictive capability, with predicted values aligning along the 1:1 reference line and an overall R^2 of 0.9726. This result indicates that the cumulative OAV of odor active compounds can serve as a basis for predicting sensory aroma perception in wine.

However, substantial variation in prediction accuracy was observed among the six sensory attributes (Fig. 7C). “Spicy” ($R^2 = 0.864$) and “green” ($R^2 = 0.837$) showed the highest predictive accuracy, indicating that these two aroma attributes are almost entirely explained by the OAV dimensions selected in this study, and they can be considered compound driven aroma dimensions. “Spicy” was highly correlated with esters such as isoamyl acetate and ethyl hexanoate (Fig. 7D), while “green” was mainly associated with C6 alcohols including 1-hexanol and (Z)-3-hexen-1-ol. Notably, (Z)-3-hexen-1-ol exhibited a strong negative correlation ($r = -0.88$) with the spicy dimension, suggesting that C6 alcohols, based on their concentration and chemical identity, may activate different combinations of olfactory receptors and thus contribute differentially to distinct sensory attributes.

Woody ($R^2 = 0.712$), red fruit ($R^2 = 0.641$) and black fruit ($R^2 = 0.617$) showed moderate predictive accuracy. “Woody” was highly correlated with methyl salicylate, 1-decanol and ethyl hexanoate. “Black fruit” was associated with β -damascenone, isoamyl alcohol and decanal, together constructing an integrated perception of black fruit notes involving blackberry, blackcurrant and plum. “Red fruit” correlated with decanal, (Z)-3-hexen-1-ol and ethyl octanoate. In contrast, floral ($R^2 = 0.300$) exhibited significantly lower predictive accuracy. It is assumed that floral notes result from the synergistic integration of low concentration terpenes (such as linalool and geraniol) and norisoprenoids (such as β -damascenone).

Even when each compound is below its own threshold, together they produce a perceived floral aroma via receptor synergy or central olfactory integration.

Moreover, variable importance in projection (VIP) analysis revealed the relative importance of different sensory attributes in discriminating the tested wines (Fig. 7E). Spicy (VIP = 0.99) and green (VIP = 0.98) had the highest Y-VIP scores, indicating that these two compound-driven attributes were the primary dimensions driving sensory differentiation among treatments. Woody (VIP = 0.94), red fruit (VIP = 0.92) and black fruit (VIP = 0.92) showed intermediate values, whereas floral (VIP = 0.82) was the lowest.

Based on the VIP results, residual analysis was then performed to examine where the OAV-PLS model systematically over or under estimated sensory perception. The residual analysis revealed systematic bias patterns for specific aroma dimensions under different inoculation strategies (Fig. 7F). O1L4 showed positive residuals for several dimensions, especially spicy and red fruit. Considering the three-species co-existence, it is therefore speculated that a high proportion of *L. plantarum* in the mixed culture, against the background of yeast-derived precursors, may produce attribute enhancement effects through the following mechanisms: (1) enhanced esterase activity leading to increased release of fruity ethyl esters such as ethyl butyrate and ethyl hexanoate; (2) up-regulated alcohol dehydrogenase pathways resulting in active metabolism of C6-C10 alcohols and aldehydes; and (3) synergistic interactions among multiple volatile components generating an integrated perception at the olfactory level that exceeds the arithmetic sum of individual OAVs.

LP exhibited a significant negative residual for the black fruit dimension, indicating that the OAV model overestimated the black fruit intensity of this wine. In this single-strain LAB fermentation, the deviation may be due to the fact that the OAVs of isoamyl alcohol and 2-phenylethanol in LP were substantially lower than those in O1L1, and these two compounds contribute indirectly to overall fruitiness through perceptual blending. O4L1 showed negative residuals for green and black fruit, suggesting that a high proportion of *O. oeni* may inhibit the synthesis of certain green related alcohols and fruity esters. These results indicate that inoculation strategies involving an initial high ratio of *O. oeni* within the three-species ecosystem may lead to an overall decrease in aroma intensity. O1L1 had the smallest residuals for most dimensions, further supporting the notion that the co-inoculation strategy with *O. oeni* and *L. plantarum* at a 1:1 ratio, in the presence of *S. cerevisiae*, achieves complementarity and synergy across all three microorganisms.

Overall, this study establishes a framework for understanding how co-culture ratios of *L. plantarum* and *O. oeni*, in combination with *S. cerevisiae*, collectively shape fermentation kinetics, bacterial community succession, volatile composition, and sensory outcomes. Mixed-LAB inoculations accelerated MLF relative to *O. oeni* monoculture. O1L1 resulted in the most balanced and complex aromatic profile, characterized by elevated concentrations of key fruity esters and terpenes, which ultimately contributed to the highest sensory scores. These findings demonstrate that strategic manipulation of LAB inoculation ratios can serve as a practical tool for modulating wine style and quality. Future research should focus on exploring the molecular mechanisms underlying interspecies interactions during co-fermentation, with the aim of developing specific starter cultures for targeted oenological applications.

3. Materials and Methods

Grapes and microorganisms

Cabernet Cabernet Gernischt grapes were harvested at commercial maturity during the 2024 vintage. The yeast strain used was *S. cerevisiae* EC1118 (Lallemand Inc., Montreal, Canada). Two lactic acid bacteria strains were employed including a commercial *O. oeni* Viniflora® Oenos (Chr. Hansen A/S, Hørsholm, Denmark) and an indigenous *L. plantarum* strain which was stored in the laboratory of the College of Food Engineering, Ludong University.

Wine production

Before fermentation, the grape must was pressed and treated with 20 mg/L pectinase and 50 mg/L sodium metabisulfite. Sucrose was added to adjust the total sugar concentration to 210 g/L. Fermentation was initiated by inoculating 250 mg/L of active dry yeast according to the manufacturer's instructions.

For the preparation of LAB inocula, *O. oeni* and *L. plantarum* were pre-cultured separately. *O. oeni* was cultivated in ATB medium at 25 °C for 60 h, while *L. plantarum* was in MRS medium for 12 h. Cells were harvested by centrifugation at 10000 × g for 15 min, washed twice with sterile saline solution (0.85% NaCl), and resuspended in the same solution to obtain a final concentration of 10⁸ CFU/mL. For mixed LAB treatments, the two bacterial suspensions were combined at the designated ratios immediately prior to inoculation.

MLF was initiated 48 h after yeast inoculation by introducing either pure or mixed LAB cultures into the fermenting must, establishing concurrent AF and MLF. Five inoculation treatments were applied (Fig. 1), coded as follows: (1) OO: MLF with *O. oeni* alone; (2) LP: MLF with *L. plantarum* alone; (3) O1L1: co-inoculation of *O. oeni* and *L. plantarum* at 1:1 ratio; (4) O1L4: co-inoculation of *O. oeni* and *L. plantarum* at 1:4 ratio; (5) O4L1: co-inoculation of *O. oeni* and *L. plantarum* at 4:1 ratio. After eight days of fermentation, yeast cells were removed by filtration, while malolactic fermentation continued under the same conditions until completion.

All fermentations were carried out at 25 °C in 5-L stainless steel vessels in triplicate. Sugar concentration during AF was daily monitored using Fehling's titration method until it decreased below 4 g/L. The concentration of L-malic acid was monitored every two days using a MA-W96-N 1620 enzymatic assay kit (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) until it fell below 0.5 g/L. Upon completion of fermentation, all wine samples were centrifuged (12000×g, 10 min) to remove cells and suspended solids, and the wines were stored at 4 °C prior to further analysis.

Yeast counts

To monitor the proliferation of yeast during AF, sampling was conducted daily under aseptic conditions and 2 mL of fermenting must was collected from the fermentation vessel. This sample was immediately analyzed using the standard plate count method. Briefly, serial dilutions of the sample were prepared in sterile saline (0.85% NaCl). Appropriate dilutions were spread-plated in duplicate onto YPD agar. The plates were incubated aerobically at 28 °C for 3-5 days, after which colony-forming units were enumerated and expressed as CFU/mL.

Next-generation sequencing

For bacterial community analysis, samples were collected at three time points during fermentation, i.e., day 3 (early phase), day 8 or 9 (mid phase), and day 12 or 14 (late phase), following yeast inoculation. Total genomic DNA was extracted from each sample using the E.Z.N.A.™ Mag-Bind Soil DNA Kit (OMEGA, USA). The extracted DNA was then sent to Sangon Biotech (Shanghai, China) for library preparation and sequencing. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified using the primer pair 341F/805R, which incorporated Illumina adapter sequences. Paired-end sequencing (2 × 300 bp) was subsequently carried out on an Illumina MiSeq platform.

The raw sequencing data, generated in FASTQ format, were processed through a series of bioinformatic steps. Cutadapt was used to trim primer and adapter sequences, followed by assembly of paired-end reads using Pear. Low-quality sequences were filtered out with Prinseq, and chimeric sequences were identified and removed using Uchime. The remaining high-quality, non-chimeric sequences were then dereplicated and clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using Usearch. Taxonomic assignment of each OTU was performed by querying its representative sequence against the Ribosomal Database Project (RDP) classifier, with a confidence threshold set at 0.80. Finally, alpha-diversity indices, including Shannon, Chao1, and Simpson, were calculated from the OTU tables to evaluate the richness and diversity of bacterial communities within individual sample.

Analytical method

The physical and chemical parameters including total reducing sugars, total acidity (calculated as g/L of tartaric acid), pH, ethanol and volatile acidity (expressed as g/L of acetic acid) and total soluble solids, were determined according to the OIV official methods²⁷.

Determination of volatiles by HS-SPME coupled with GC×GC-MS

Volatile compounds were extracted using headspace solid-phase microextraction (HS-SPME). For each sample, 5 mL wine was placed in a 20 mL headspace vial along with an internal standard solution of 3-octanol (100 mg/L in ethanol) and saturated with 1.5 g sodium chloride. The vial was sealed with a silicone septum and equilibrated in a water bath at 50 °C for 10 min. Volatiles were extracted using a 50/30 µm DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA, USA) exposed to the headspace for 30 min at 50 °C, followed by thermal desorption in the GC injector at 250 °C for 5 min. All analyses were performed in triplicate.

The Two-Dimensional Gas Chromatography-Mass Spectrometry (GC×GC-MS) analysis was conducted on a system consisting of a GCMS-QP2020NX mass spectrometer coupled to a GC-2030 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a two-stage quad-jet thermal modulator. Separation was achieved using a primary column (SH-I-5SiL MS, 30 m × 0.25 mm, 0.25 µm) and a secondary column (SH-17 MS, 1.2 m × 0.18 mm, 0.18 µm). Helium (99.999%) was used as the carrier gas at a rate of 1 mL/min. The injector was operated in splitless mode at 250 °C. The primary oven temperature was programmed as follows: held at 40 °C for 2 min, increased to 230 °C at 3 °C/min, and held for 5 min. The secondary oven followed an identical temperature program with an offset of +5 °C. The modulator had a period of 5 s, with the hot jet temperature set at 70 °C and the cold jet at 9 °C.

Mass spectrometry detection was performed in electron ionization (EI) mode at 70 eV. The ion source temperature was 200 °C, the transfer line was maintained at 250 °C, and the mass scan range was m/z 35–450 at a scan speed of 20,000 u/s.

Compound identification was based on comparison of mass spectra with the NIST20 library and confirmed by calculating retention indices (RI) using a C7-C40 alkane series analyzed under identical conditions. Semi-quantification was performed by normalizing the peak area of each compound to that of the internal standard (3-octanol).

Sensory evaluation and ethics statement

Sensory analysis

Descriptive sensory analysis was performed by a panel of 16 assessors (11 females and 5 males, aged from 21 to 48). Prior to the formal sessions, panelists completed a two-week training program in accordance with the ISO 8586:2012 standard, comprising five one-hour sessions aimed to agree upon a common vocabulary for aroma attributes and to calibrate the intensity grading system. The defined attributes included red fruit, black fruit, floral, spicy, herbaceous, green, and an overall quality impression.

During the evaluation, 25 mL wine samples were served in standard ISO 3591:1997 wine-tasting glasses at 20 °C under controlled sensory booth conditions. Each attribute was scored on a structured 9-point intensity scale where 0 indicated not perceived and 9 represented extremely intense. Samples were presented in a randomized order using three-digit codes to minimize serving-order bias. Each wine was evaluated in duplicate across two independent tasting sessions.

Ethics statement

All sensory evaluation procedures involving human participants were conducted in strict accordance with the ethical principles of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board (IRB) / Ethics Committee of Ludong University (Approval No. LDU-IRB2026051002).

The study was performed following appropriate protocols for protecting the rights and privacy of all participants. Participants were fully informed of the objectives, procedures, potential risks, and benefits of the study prior to their involvement. Written informed consent was obtained from each participant before the commencement of any sensory assessment. Participants were informed that their involvement was entirely voluntary and that they could withdraw from the study at any time without penalty. All personal data collected during the sensory sessions were anonymized and handled in compliance with the General Data Protection Regulation and relevant national data protection laws, ensuring the confidentiality and privacy of all participants.

Statistical analysis

Statistical analysis was performed using SPSS 26 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was applied to compare the concentrations of volatile compounds across different fermentation treatments, with statistical significance set at $p < 0.05$. Principal component analysis (PCA) was conducted on the volatile compounds obtained from HS-GC×GC-MS to visualize sample clustering and inter-variable relationships in a reduced dimension. To elucidate the correlation between sensory attributes and volatile compounds, partial least squares regression (PLS-R) was carried out using SIMCA 14.1 software (Umetrics, Umeå, Sweden).

Declarations

Acknowledges

We are grateful to the National Natural Science Foundation of China (General program, No. 32272281), the Science and Technology Innovation Development Program of Yantai (No. 2024JCYJ068), Key Project of Teaching Reform Research in Universities of Shandong Province (No. Z2023055), Yinchuan Science and Technology Innovation Team Program (YCHT2026041) and Industrial Leading Talents of Yantai's "Double-Hundred Plan" (2025076).

Credit authorship contribution statement

Yaxin Cao: Wrote the original draft. Shuyang Sun: Designed the experiments and revise the draft. Ziruo Sun and Taihong Liu: Performed the experiments. Wenguang Jiang: Conceptualised the idea and edited the paper.

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. And the authors have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

References

1. Zhang, S., Xing, X., Chu, Q., Sun, S. & Wang, P. Impact of co-culture of *Lactobacillus plantarum* and *Oenococcus oeni* at different ratios on malolactic fermentation, volatile and sensory characteristics of mulberry wine. *LWT* 169, 113995 (2022).
2. Paramithiotis, S., Stasinou, V., Tzamourani, A., Kotseridis, Y. & Dimopoulou, M. Malolactic fermentation—Theoretical advances and practical considerations. *Fermentation* 8, 521 (2022).
3. Bajer, T., Šmejda, P., Kubálek, J. & Bajerová, P. Comparative analysis of aroma profiles and determination of key constituents in organically and conventionally produced white wines. *npj Sci. Food* 10, 166 (2026).

4. Virdis, C., Sumby, K., Bartowsky, E. & Jiranek, V. Lactic acid bacteria in wine: Technological advances and evaluation of their functional role. *Front. Microbiol.* 11 (2021).
5. Balmaseda, A., Rozès, N., Bordons, A. & Reguant, C. Characterization of malolactic fermentation by *Lactiplantibacillus plantarum* in red grape must. *LWT* 199, 116070 (2024).
6. Liu, K. et al. Enhancing blueberry wine quality and antioxidant capacity through mixed fermentation with *S. cerevisiae* and *O. oeni*. *npj Sci. Food* 9, 43 (2025).
7. Pan, Y., Yin, X., Zhang, J., Bai, W. & Sun, J. Effect of co-fermentation of yeast and lactic acid bacteria on color of mulberry wine. *Food Biosci.* 76, 108220 (2026).
8. Silva, A. et al. Tailoring yeast and bacterial consortia to modulate wine fermentation profiles. *Food Res. Int.* 229, 118486 (2026).
9. Devi, A. & Anu-Appaiah, K. A. Mixed malolactic co-culture (*Lactobacillus plantarum* and *Oenococcus oeni*) with compatible *Saccharomyces* influences the polyphenolic, volatile and sensory profile of Shiraz wine. *LWT* 135, 110246 (2021).
10. Devi, A., Anu-Appaiah, K. A. & Lin, T. F. Timing of inoculation of *Oenococcus oeni* and *Lactobacillus plantarum* in mixed malo-lactic culture along with compatible native yeast influences the polyphenolic, volatile and sensory profile of the Shiraz wines. *LWT* 158, 113130 (2022).
11. Zhang, B. et al. Impact of indigenous *Oenococcus oeni* and *Lactiplantibacillus plantarum* species co-culture on *Cabernet Sauvignon* wine malolactic fermentation: Kinetic parameters, color and aroma. *Food Chem. X* 22, 101369 (2024).
12. Liu, P. et al. Processing technology as aroma architect: OAV fingerprints decode differentiation and compatibility of key odorants in Fuding Dabai tea via GC×GC-TOF-MS and sensomics. *Food Chem. X* 34, 103530 (2026).
13. Lin, M. X. et al. *Lactiplantibacillus plantarum* in fermented beverages: Properties, mechanisms, and future prospects. *J. Funct. Foods* 129, 106864 (2025).
14. Mayo, B. & Flórez, A. B. in *Encyclopedia of Dairy Sciences* (3rd edn) (eds McSweeney, P. L. H. & McNamara, J. P.) 206–217 (Academic Press, 2022).
15. Yuan, Y., Li, Y., Meng, Q., Liu, S. & Shi, K. Impact of *trpB* and *trpA* on acid stress response and malolactic fermentation in *Lactiplantibacillus plantarum* XJ25. *Food Biosci.* 75, 108222 (2026).
16. Zhao, Y. et al. The *AI-2/LuxS* quorum sensing system enhances stress tolerance and enological performance of *Lactiplantibacillus plantarum* during malolactic fermentation. *Food Microbiol.* 134, 104923 (2026).
17. Seddik, H. A. et al. *Lactobacillus plantarum* and its probiotic and food potentialities. *Probiotics Antimicrob. Proteins* 9, 111–122 (2017).
18. Scott, W. T., Henriques, D., Smid, E. J., Notebaart, R. A. & Balsa-Canto, E. Dynamic genome-scale modeling of *Saccharomyces cerevisiae* unravels mechanisms for ester formation during alcoholic fermentation. *Biotechnol. Bioeng.* 120, 1998–2012 (2023).
19. Kaushik, S., Hung, T. & Chang, C. E. A. Molecular mechanics studies of factors affecting overall rate in cascade reactions: Multi-enzyme colocalization and environment. *Protein Sci.* 33, 5175 (2024).
20. Song, J. et al. From fruit to wine: impact of fig variety and peeling on fig wine characteristics. *npj Sci. Food* 9, 284 (2025).
21. Brizuela, N. S. et al. Advantages of using blend cultures of native *L. plantarum* and *O. oeni* strains to induce malolactic fermentation of Patagonian Malbec wine. *Front. Microbiol.* 9 (2018).
22. Brizuela, N. S., Bravo-Ferrada, B. M., Pozo-Bayón, M. Á., Semorile, L. & Tymczynszyn, E. Changes in the volatile profile of Pinot noir wines caused by Patagonian *Lactobacillus plantarum* and *Oenococcus oeni* strains. *Food Res. Int.* 106, 22–28 (2018).
23. Costello, P. J., Siebert, T. E., Solomon, M. R. & Bartowsky, E. J. Synthesis of fruity ethyl esters by acyl coenzyme A: alcohol acyltransferase and reverse esterase activities in *Oenococcus oeni* and *Lactobacillus plantarum*. *J. Appl. Microbiol.* 114, 797–806 (2013).
24. Brizuela, N. et al. *Lactobacillus plantarum* as a malolactic starter culture in winemaking: A new (old) player? *Electron. J. Biotechnol.* 38, 10–18 (2019).
25. Michlmayr, H. et al. Release of wine monoterpenes from natural precursors by glycosidases from *Oenococcus oeni*. *Food Chem.* 135, 80–87 (2012).
26. Cappello, M. S., Zapparoli, G., Logrieco, A. & Bartowsky, E. J. Linking wine lactic acid bacteria diversity with wine aroma and flavour. *Int. J. Food Microbiol.* 243, 16–27 (2017).
27. OIV. *Compendium of International Methods of Wine and Must Analysis* (2 vols) (OIV, 2025).
28. Kong, C.-L. et al. Flavor modification of dry red wine from Chinese spine grape by mixed fermentation with *Pichia fermentans* and *S. cerevisiae*. *LWT* 109, 83–92 (2019).
29. Karadimou, C., Kalogiouri, N. P., Petropoulos, S. & Koundouras, S. Development and validation of a headspace solid-phase microextraction (HS-SPME) method coupled with gas chromatography–ion trap mass spectrometry (GC–IT–MS) for determining both free and bound volatile compounds in Greek wines belonging to the Mandilaria cultivar (*Vitis vinifera* L.). *Microchem. J.* 216, 114734 (2025).
30. Cai, J. et al. Influence of pre-fermentation cold maceration treatment on aroma compounds of *Cabernet Sauvignon* wines fermented in different industrial scale fermenters. *Food Chem.* 154, 217–229 (2014).

31. Gao, F. et al. Preliminary characterization of chemical and sensory attributes for grapes and wines of different cultivars from the Weibei Plateau region in China. *Food Chem. X* 21, 101091 (2024).

32. Wang, M. et al. The dominance of commercial ADYs and their correlation between fungal diversity, volatile compounds during industrial fermentations of *Cabernet Sauvignon* wines. *Food Chem. X* 29, 102725 (2025).

33. Liu, Q. et al. From microbial communities to aroma profiles: A comparative study of spontaneous fermentation in merlot and cabernet sauvignon wines. *Food Chem. X* 26, 102317 (2025).

Tables

Table 1. Basic composition of wines resulting from different treatments

Parameters*	LP	OO	O4L1	O1L1	O1L4
Alcohol content (% v/v)	12±0.02d	11.6±0.05b	11.5±0.03a	12.1±0.02e	11.8±0.04c
Reducing sugar (g/L)	1.44±0.02b	3.2±0.03e	3.01±0.02d	0.9±0.01a	2.03±0.02c
Total acidity (g/L)	5.04±0.05a	4.95±0.03a	5.07±0.03a	5.01±0.02a	5.13±0.04a
Volatile acid (g/L)	0.41±0.01a	0.36±0.02a	0.46±0.02a	0.43±0.01a	0.42±0.02a
pH	3.66±0.02a	3.68±0.02a	3.63±0.03a	3.65±0.03a	3.62±0.03a
Soluble solids (%)	8.06±0.05ab	7.95±0.05a	8.11±0.05b	7.99±0.05a	8.17±0.05c

*Different superscript letters within a row denote significant differences ($p < 0.05$) among wine samples.

Table 2. OAV analysis of volatile compounds identified in wines from five treatments

Compound	Threshold (µg/L)	Description	OO	LP	O4L1	O1L1	O1L4	Reference
Ethyl acetate*	7500	Fruity	1.33±0.02a	1.62±0.02d	1.37±0.02a	1.43±0.03b	1.48±0.03c	28
Ethyl butyrate	20	Strawberry, apple, banana	16.12±0.59b	16.65±0.52b	14.66±0.36a	20.23±0.48d	17.91±0.33c	28
Isoamyl acetate	30	Banana	0.87±0.03c	0.59±0.03b	0.87±0.03c	0.52±0.02a	0.57±0.02b	28
Ethyl hexanoate	5	Flowery, fruity	67.84±4.80a	70.49±5.22ab	72.85±4.42ab	81.72±7.00bc	84.63±6.70c	28
Ethyl heptanoate	220	Pineapple, fruit	0.29±0.03b	0.27±0.02ab	0.21±0.01a	0.26±0.01ab	0.24±0.01ab	28
Ethyl 2-hexenoate	0.02	Spicy, candy, earthy	48.50±1.00c	37.00±0.50a	51.00±0.50d	40.50±0.50b	47.00±1.00c	29
Ethyl octanoate	580	Sweet, floral, fruity, banana, pear	0.29±0.03a	0.28±0.03a	0.36±0.02b	0.32±0.02a	0.35±0.03b	30
Ethyl decanoate	200	Fruity, rose	5.98±0.47ab	6.51±0.42b	5.39±0.39a	6.80±0.46b	6.33±0.47b	28
Methyl salicylate	40	Mint	0.52±0.04b	0.45±0.03a	0.41±0.01a	0.43±0.02a	0.43±0.04a	31
Ethyl dodecanoate	1500	Oily, fatty, fruity	0.15±0.01b	0.19±0.01c	0.20±0.01c	0.13±0.01a	0.15±0.01b	30
Ethyl 3-methylbutanoate	3	Banana, sweet fruit	29.54±2.26a	40.78±3.02c	30.37±1.78a	37.41±3.51b		28
Isobutanol	40000	Alcohol, wine, nail polish	0.26±0.00c	0.28±0.00d	0.25±0.00b	0.22±0.00a	0.28±0.00d	32
Isoamyl alcohol	30000	Whiskey, nail polish	1.04±0.01d	0.95±0.01c	0.64±0.00a	0.98±0.01c	0.91±0.01b	28
1-Hexanol	8000	Green, grass	0.18±0.01b	0.17±0.01ab	0.15±0.01a	0.14±0.01a	0.16±0.01a	28
(Z)-3-Hexen-1-ol	400	Green, cypress	0.11±0.01a	0.14±0.01b	0.22±0.00d	0.24±0.0e	0.17±0.01c	28
(Z)-2-Hexen-1-ol	400	Herbaceous, green	0.23±0.01a	0.30±0.03b	0.30±0.02b	0.27±0.02b	0.24±0.01a	30
1-Heptanol	250	Oily	0.60±0.05a	0.62±0.05a	0.68±0.04ab	0.73±0.05b	0.54±0.04a	30
2-Ethyl-1-hexanol	10	Sweet, faint floral	8.64±0.89bc	7.60±0.35ab	9.18±0.88c	7.04±0.32a	8.37±0.58bc	33
2-Nonanol	75	Fruity, mouldy, musty, mushroom	0.26±0.01bc	0.30±0.02c	0.21±0.01a	0.25±0.01ab	0.26±0.04bc	30
1-Octanol	900	Intense citrus, rose	0.21±0.02bc	0.16±0.01a	0.23±0.02c	0.14±0.02a	0.18±0.01b	28
1-Nonanol	600	Honey, sweet	0.19±0.02ab	0.18±0.02a	0.22±0.02b	0.26±0.03c	0.19±0.01ab	33
1-Decanol	400	Orange flowery	0.27±0.01b	0.24±0.01ab	0.23±0.02a	0.20±0.01a	0.25±0.02ab	28
Benzyl alcohol	100	Floral	1.04±0.10bc	0.96±0.07ab	0.83±0.03a	0.98±0.08ab	1.07±0.09c	29
2-Phenylethanol	14000	Rose, honey	1.34±0.01c	1.07±0.01b	0.99±0.01a	1.27±0.01c	1.48±0.01d	30
Butyric acid	173	Cheese, cream	2.24±0.13ab	2.46±0.17b	1.95±0.12a	2.50±0.18b	2.18±0.11ab	33

Hexanoic acid	420	Cheese, fatty	2.23±0.19ab	2.48±0.20b	2.99±0.23c	2.83±0.17c	2.07±0.13a	30
Octanoic acid	500	Rancid, cheese, fatty acid	2.10±0.18a	1.90±0.15a	2.54±0.20b	1.96±0.15a	2.17±0.18a	30
Decanal	2	Soap, waxy, fatty	6.75±0.51ab	7.63±0.62bc	5.72±0.44a	9.29±0.79d	8.23±0.40cd	33
Linalool	15	Flower, fruity, Muscat	0.25±0.00a	0.36±0.00d	0.30±0.00b	0.31±0.00c	0.33±0.00c	28
Geraniol	20	Citric, geranium	2.57±0.20b	2.15±0.15a	2.37±0.07ab	2.68±0.13b	2.25±0.13a	30
4-Terpineol	250	Flowery, orange juice	0.14±0.01a	0.21±0.01b	0.17±0.01ab	0.15±0.01a	0.19±0.01ab	28
β-Damascenone	0.05	Apple, rose, honey	38.60±0.20c	35.60±0.40a	36.80±0.20b	42.20±0.40e	41.00±0.20d	29

*Different superscript letters within a row denote significant differences ($p < 0.05$) among wine samples

Figures

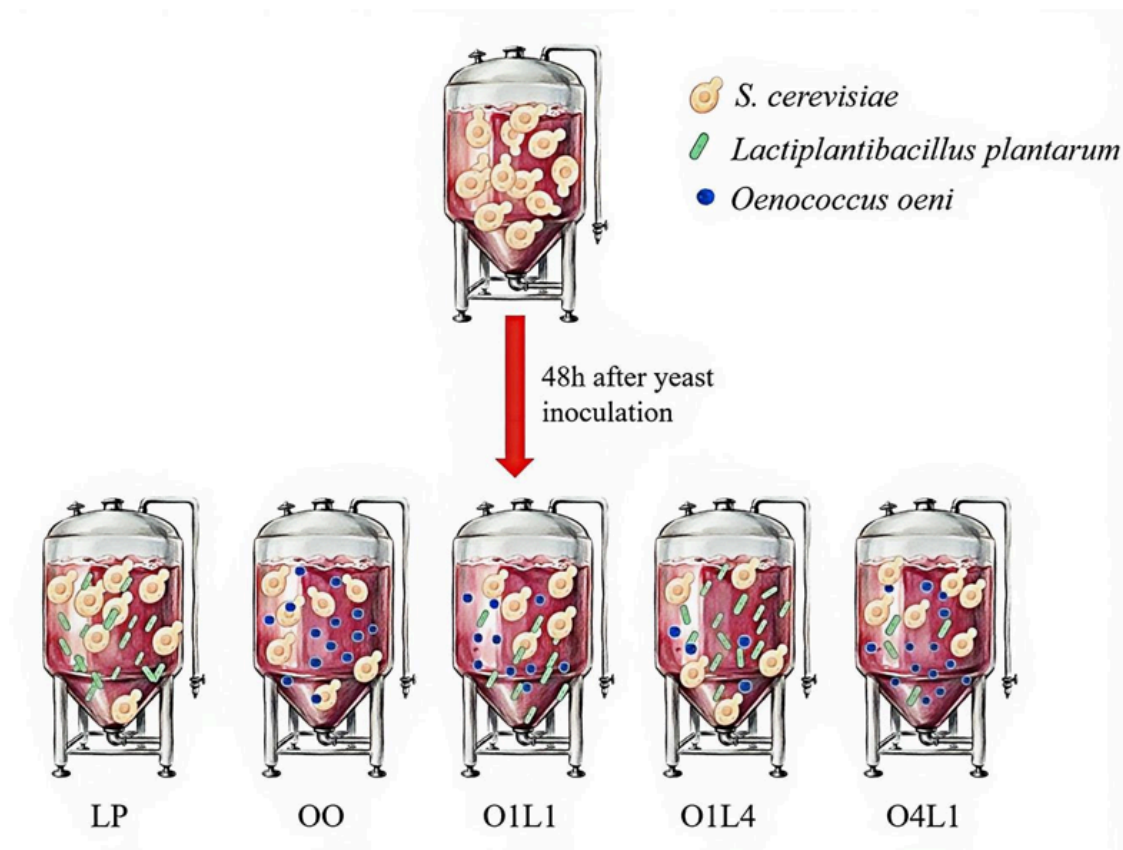


Figure 1

Schematic diagram of experimental design

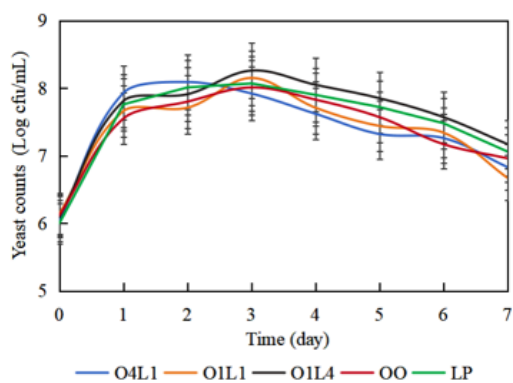


Fig. 2A

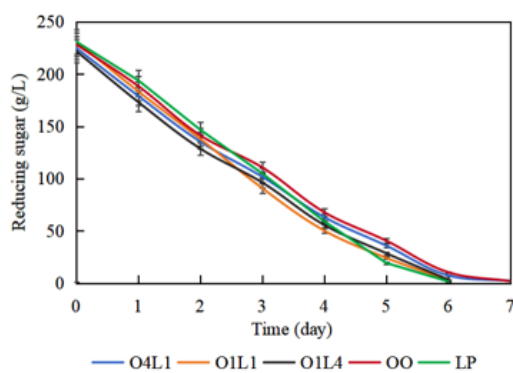


Fig. 2B

Figure 2

2A. Yeast counts during fermentation resulting from different treatments.

2B. Consumption of reducing sugar during fermentation.

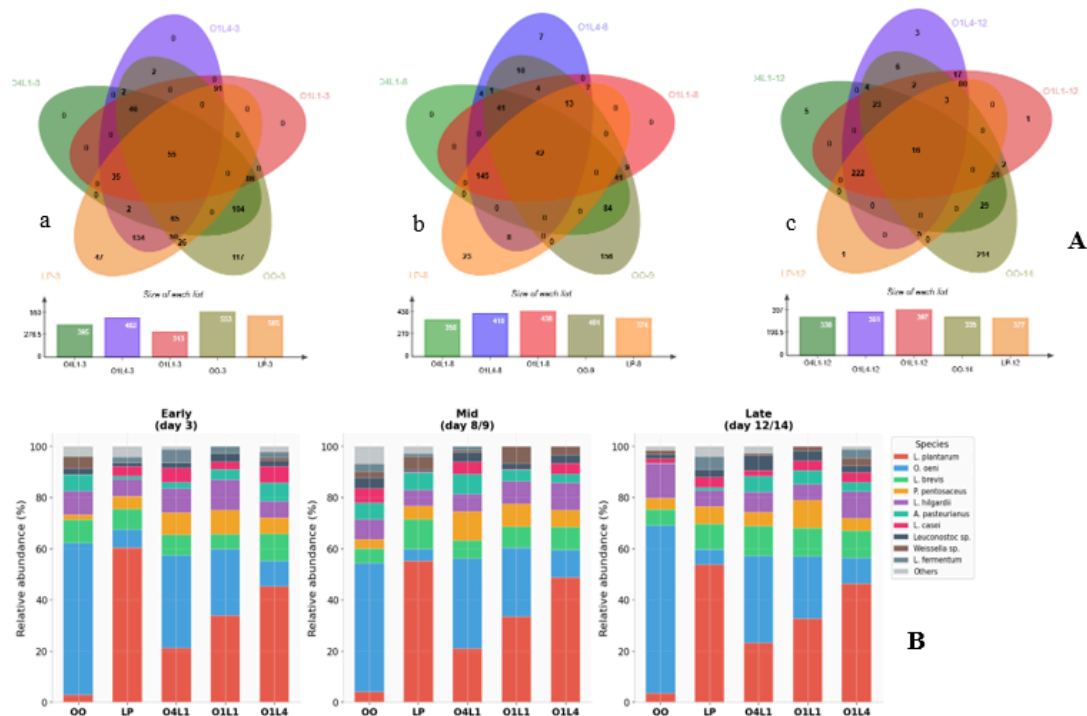


Figure 3

Plots of microbial diversity during fermentation (A) at early (day 3, a), middle (day 8 or 9, b), and late (day 12 or 14, c) phases. Bacterial community composition (B) during at early, middle and late phases.

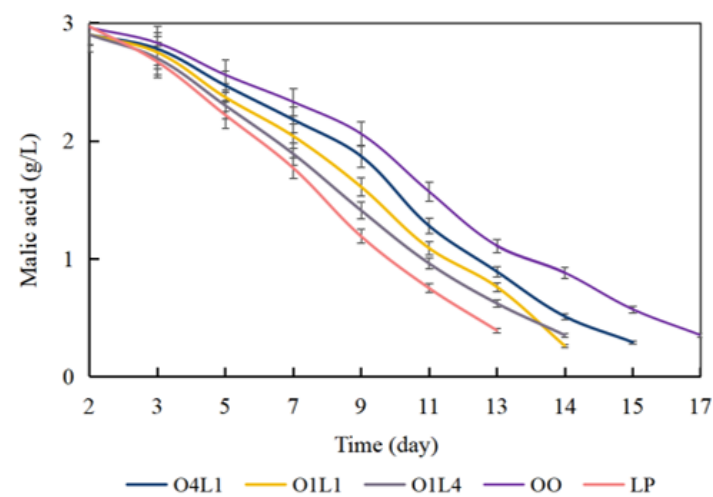


Figure 4

Degradation of L-malic acid during fermentation.

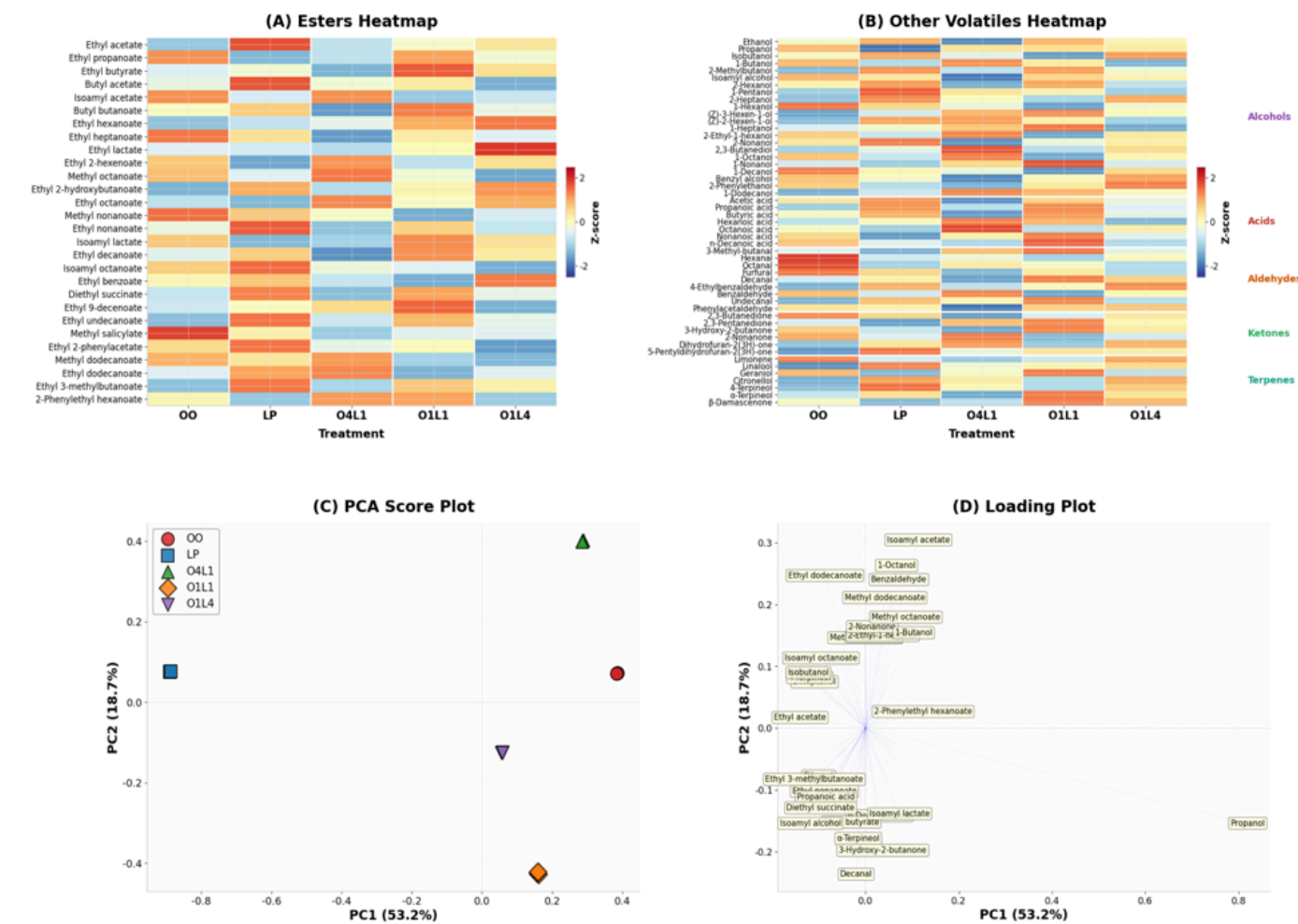


Figure 5

Page 15/17

Multivariate analysis of volatile compounds in wines. (A) Heatmap of esters (Z-score normalized). (B) Heatmap of other volatile compounds (Z-score normalized). (C) PCA score plot based on all volatile compounds. Ellipses represent 95% confidence intervals. (D) PCA loading plot.

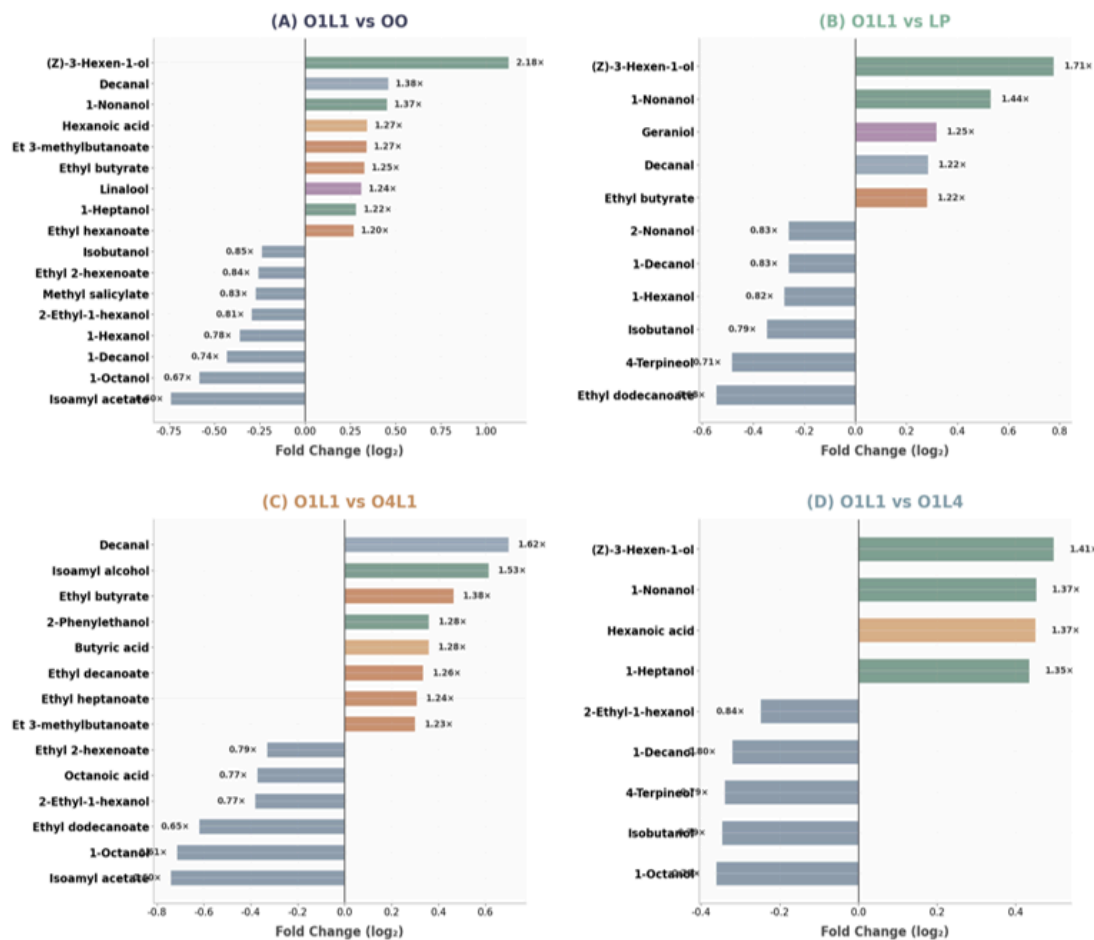


Figure 6

Fold change analysis of OAV-active volatile compounds in wines from co-inoculation treatments relative to O1L1. Pairwise comparisons: (A) O1L1 vs. OO; (B) O1L1 vs. LP; (C) O1L1 vs. O4L1; (D) O1L1 vs. O1L4.

Note: Compounds with fold change > 1.2 or < 0.85 relative to the comparator are shown.

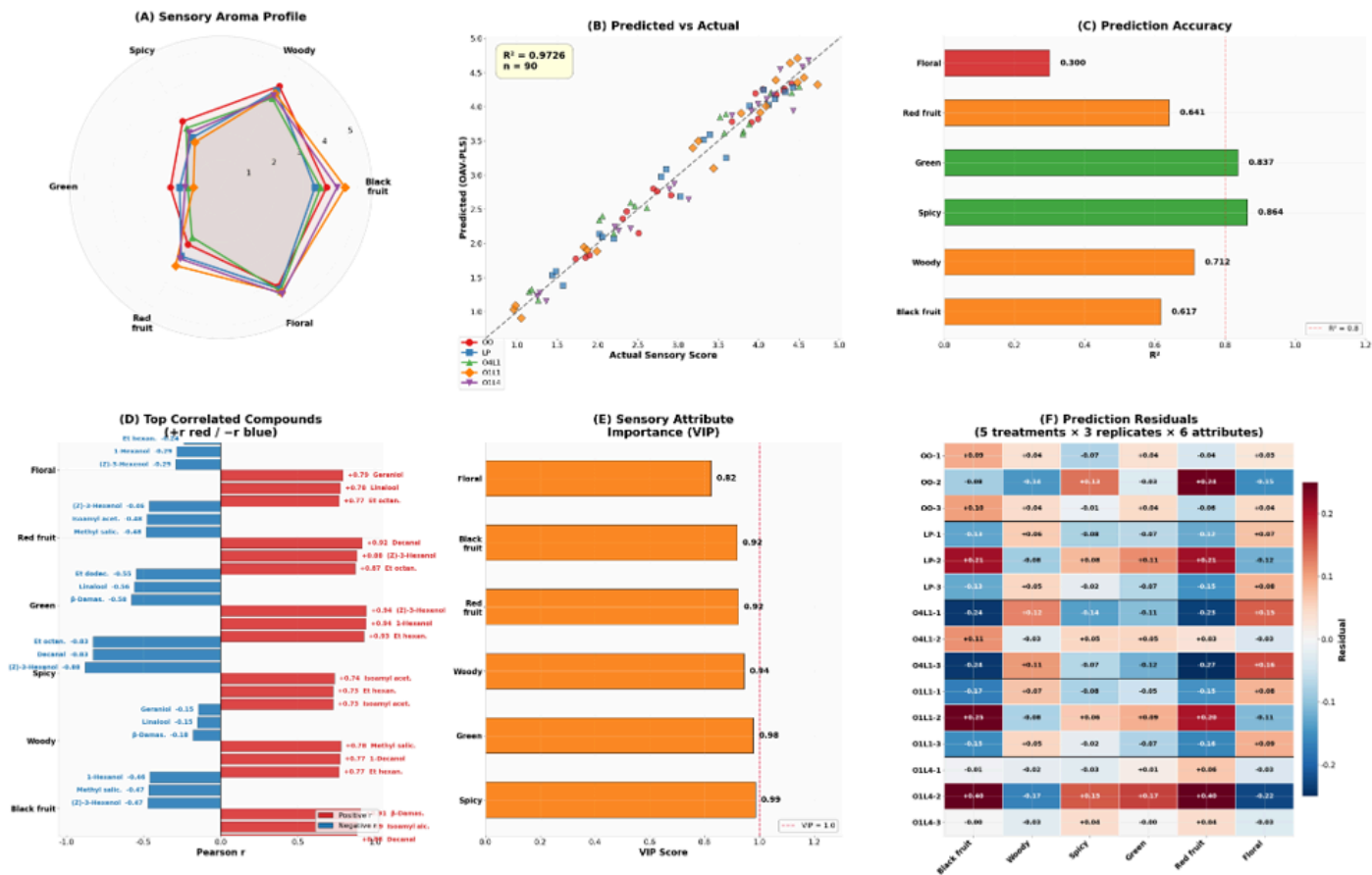


Figure 7

Sensory evaluation and OAV-PLS analysis validation of wines from different fermentations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.docx