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Acid management strategies in red wines from a coastal Mediterranean climate in California

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ABSTRACT

Nebbiolo and Tannat wines from the Central Coast of California were produced using three different acid management strategies in addition to a control (i), namely: (ii) blocking malolactic fermentation (MLFB), (iii) inoculation with the non-*Saccharomyces* lactic acid-producing yeast *Lachancea thermotolerans* (Lachancea), and (iv) treatment with ion exchange (IE) followed by blending at a 30 % proportion. Ion exchange lowered pH by 0.31 units relative to control wines, and reduced cation concentrations, specifically potassium and calcium, by 9 % and 13 % compared to the control, respectively. MLFB and IE wines showed enhanced colour stability. Compared to control wines, MLFB wines had 19 % and 5 % higher tannin and total phenolics concentrations, respectively, while IE wines had 19 % and 11 % higher concentrations, respectively, and both were rated as having the highest colour saturation in Nebbiolo wines upon sensory analysis. *Lachancea thermotolerans* increased titratable acidity by 24 % through lactic acid production and had lower concentrations of tannins, polymeric pigments, and phenolics, and lower odour activity values for ethyl butyrate, ethyl isovalerate, isoamyl acetate, ethyl hexanoate, and ethyl *n*-octanoate. However, Lachancea wines showed the highest perceived acidity and the highest duration of red fruit flavour on the palate in Nebbiolo wines. All acid management strategies improved chemical and sensory outcomes compared to the control, with specific chemical and sensory features and stylistic implications for each of them.

KEYWORDS: acid management, *Lachancea thermotolerans*, ion exchange, malolactic fermentation

INTRODUCTION

Acid management is a key component of winemaking and has become increasingly relevant in the context of cyclic climate change, changing viticultural and agronomical practices, and their impact on grape composition (Aloy *et al*., 2024). Indeed, a combination of rising temperatures and viticultural practices geared towards hastening ripening (Webb *et al*., 2012) has resulted in increased sugar accumulation, decreased organic acid concentrations in grapes, and higher potassium uptake (Villette *et al*., 2020), resulting in wines with higher pH and lower titratable acidity (Dequin *et al*., 2017; Pons *et al*., 2017).

Tartaric and malic acids are the predominant organic acids in wine grapes (Volschenk *et al*., 2017). Higher temperatures during grape ripening accelerate the respiration of malic acid, leading to reduced acid concentrations at harvest and higher wine pH (Martínez-Lüscher *et al*., 2025). High pH can negatively affect wine quality. Microbial stability is compromised, as higher pH conditions favour the growth of spoilage organisms including *Pediococcus*, *Lactobacillus*, and *Brettanomyces* (Krieger-Weber *et al*., 2020; Wade *et al*., 2019; Wedral *et al*., 2010). Additionally, the antimicrobial effectiveness of sulfur dioxide (SO_2) is diminished at higher pH due to a lower proportion of its active molecular form (Payan *et al*., 2023). Wine colour is also impacted by pH, as lower pH conditions favour the flavylium cation form of anthocyanins, resulting in more intense red colour. In contrast, higher pH promotes colourless forms of anthocyanins, resulting in reduced colour stability (Forino *et al*., 2020; Gambuti *et al*., 2022). Overall, these shifts can negatively affect microbial stability (Payan *et al*., 2023), colour (Sims & Morris, 1984), and sensory characteristics, resulting in a loss of perceived freshness and alterations of wine balance (Morata *et al*., 2019). Therefore, it is important to investigate the effects of alternative acid management strategies on the chemical and sensory composition of red wines.

The simple approach of adjusting acidity via tartaric acid addition is arguably the most common acid adjustment method in contemporary winemaking. This is because tartaric acid is relatively cost-effective, simple to implement at different stages of winemaking, and its outcomes are generally more predictable than biological acidification approaches (Jackson, 2020; Payan *et al*., 2023). Tartaric acid is also the strongest naturally occurring organic acid in wine due to the low pKa of its first carboxylic group (2.98), which explains its larger impact on wine pH relative to other wine acids (Payan *et al*., 2023). However, relying on tartaric acid additions to reduce pH is inefficient due to the high pKa of its second carboxylic group (4.34) and the buffering capacity of must/wine. At a typical wine pH (3.5), most of the tartaric acid is not fully dissociated (Payan *et al*., 2023). Therefore, liberal additions of tartaric acid will contribute exceedingly more to titratable acidity and less to pH in wine, resulting in more tart wines without a proportional decrease in pH (Payan *et al*., 2023). This practice also presents limitations due to the potential of tartaric acid to precipitate out as potassium bitartrate (KHT), leading to tartrate instability (Ribéreau-Gayon, 2006). This occurs when bitartrate ions bind with potassium ions naturally present in wine, forming crystals that can negatively impact both chemical stability and consumer perception (Payan *et al*., 2023). Cold stabilisation is a common method used to mitigate tartrate precipitation. However, this process is energy-intensive, time-consuming, and costly, highlighting the need for alternative acid management strategies (Martínez-Pérez *et al*., 2020). Other methods to prevent tartaric acid precipitation include the addition of metatartaric acid, mannoproteins, and carboxymethylcellulose (CMC), among other additives. Metatartaric acid is unstable, while CMC is less effective at tartrate stability in red wines and can also impact red wine colour (Lasanta & Gómez, 2012). Mannoproteins present filterability issues in red wines (Cui *et al*., 2024). The drawbacks of these additives also stress the importance of alternative acid management strategies to the addition of tartaric acid.

Malolactic fermentation (MLF), carried out primarily by lactic acid bacteria such as *Oenococcus oeni*, impacts wine acidity by converting L-malic acid to L-lactic acid and carbon dioxide (Lonvaud-Funel, 1995). This process reduces titratable acidity and increases pH, resulting in a softer mouthfeel, likely due to the moderating effect of lower acidity on perceived astringency (Fontoin *et al*., 2008). MLF also provides microbial stabilisation of wines by removing malic acid, which acts as a carbon source for spoilage yeasts (Paramithiotis *et al*., 2022). MLF can also have positive contributions to wine aroma and complexity, with some studies showing increased ester concentrations in wines that undergo this secondary fermentation (Izquierdo Cañas *et al*., 2008; Lytra *et al*., 2020). While desirable in some wines, MLF can exacerbate the challenges associated with high pH wines. It may also alter the aromatic profile of the resulting wines through ester hydrolysis, potentially reducing fruity characteristics (Lonvaud-Funel, 1995). As a result, blocking MLF, commonly accomplished through SO_2 additions, can be employed as a strategy to preserve acidity. However, lactic acid bacteria can still cause spontaneous MLF in bottled wines. Therefore, it is important that wines that are MLF blocked receive appropriate SO_2 additions as well as sterile filtration prior to bottling to minimise the risk of refermentation in bottle (Jackson, 2020).

Biological production of acidity can be accomplished by both *Saccharomyces* and non-*Saccharomyces* yeasts. Non-*Saccharomyces* yeasts that can be used as an alternative biological acidification strategy include strains such as *Lachancea thermotolerans, Starmerella bacillaris, Candida stellata,* and bacteria such as *Lactiplantibacillus plantarum* (Vicente *et al*., 2022). *Starmerella bacillaris* is a yeast that produces large amounts, approximately 100 mg/L, of pyruvic acid due to its glycerol-pyruvic pathway preference (Vicente *et al*., 2022). Production of 2-oxoketoglutaric acid as a result of *Starmerella bacillaris* may also play a role in its acidifying ability (Vicente *et al*., 2022; Magyar *et al*., 2014). *Candida stellata* is a non-*Saccharomyces* yeast that can also

have an acidifying effect on wines due to its ability to produce succinic acid (Vicente *et al*., 2022; Ciani *et al*., 2000). *Lactiplantibacillus plantarum* is a lactic acid bacterium that produces lactic acid from both malic acid degradation and sugar metabolism, having an acidifying effect when used during alcoholic fermentation (Vicente *et al*., 2022; Onetto & Bordeu, 2015).

Lachancea thermotolerans is a non-*Saccharomyces* yeast that produces lactic acid during alcoholic fermentation via the lactate dehydrogenase pathway, leading to increased titratable acidity and reduced pH (Sgouros *et al*., 2020). Because *Lachancea thermotolerans* has limited fermentative capacity (typically 5 to 9 % *v/v* ethanol), it is typically used in sequential inoculation with *Saccharomyces cerevisiae* to complete fermentation (Morata *et al*., 2018). In addition to acidification, this yeast can influence sensory characteristics through the production of esters such as ethyl isobutyrate, which contributes to fruity, strawberry-like aromas (Benito, 2018). However, lactic acid production varies significantly by strain, ranging from 0.18 to 10 g/L (Sgouros *et al*., 2020), suggesting that the selection of the strain is crucial and pointing out the need for further research on sensory and varietal impacts caused by this yeast.

Ion exchange represents a physicochemical approach to acid management in winemaking. This technique uses negatively charged resins to exchange cations, primarily potassium (K^+), for hydrogen ions (H^+), resulting in decreased pH and increased titratable acidity (Lasanta *et al*., 2017). By reducing potassium concentrations, ion exchange also improves tartrate stability and alters the buffering capacity of wine, making pH adjustments more stable over time (Ponce *et al*., 2018). Typically, wines treated with ion exchange can show pH as low as 2 (Lasanta *et al*., 2017) and are exceedingly too acidic for direct consumption. Therefore, after treatment, treated wines are blended back into untreated wine. Further research is needed to optimise blending ratios and to evaluate their impact across different red wine varieties.

While all of these acid management strategies, namely blocking MLF, *Lachancea thermotolerans* fermentation, and ion exchange, have been studied individually, there is limited research comparing their effects within the same experimental protocol. Additionally, the impact of these strategies across different red wine varietals with distinct acid and phenolic compositions remains underexplored. Varieties such as Nebbiolo and Tannat, which differ substantially in their inherent phenolic makeup, were herein purportedly selected to understand better how acid management interacts with varietal characteristics. Furthermore, the long-term effects of these techniques during wine ageing are not well understood. Given that Nebbiolo and Tannat wines are usually intended for extended ageing, it is important to evaluate how these acidification strategies influence chemical stability and sensory properties over time. Accelerated ageing studies offer a practical approach to addressing this gap.

The objective of the present study was to compare the effects of three acid management strategies, including blocking MLF, fermentation with *Lachancea thermotolerans*, and ion exchange, on the chemical composition and sensory characteristics of two contrastingly different red wine varietals, Nebbiolo and Tannat, grown in a California coastal Mediterranean climate. Additionally, this study aimed to evaluate the stability of these treatments under accelerated ageing conditions to understand their long-term implications for wine quality better.

MATERIALS AND METHODS

1. Winemaking

Nebbiolo grapes (1.26 tons) were harvested from Mora Vineyard (Santa Ynez AVA, CA, USA) on September 26, 2024. Tannat grapes (1.46 tons) were harvested from Sunnybrook Vineyard (Paso Robles AVA, CA, USA) on October 10, 2024. For both varietals, grapes were transported to the Justin and Jerry Lohr Centre for Wine and Viticulture (Cal Poly), where they were manually sorted (Armbruster, Güglingen-Frauenzimmern, Germany), destemmed (Armbruster, Güglingen-Frauenzimmern, Germany) and pumped (Waukesha, Carlsen Associates, Healdsburg, CA, USA) into twelve 110-litre stainless-steel fermenter tanks (Westec, Healdsburg, CA, USA). Each fermenter was filled with 80 kg of must and received 30 mg/L of SO_2. Tanks were randomly assigned to one of four treatments: control, malolactic fermentation blocked (MLFB), inoculation with *Lachancea thermotolerans* (Lachancea), and ion exchange treatment of finished wine followed by blending at a 30 % proportion (IE). All treatments were performed in triplicate. Due to higher Brix levels in Tannat grapes (29 Brix), their corresponding musts were ameliorated with a 12 % acidified water addition (3 g/L tartaric acid) to target 25.0 Brix. Control, MLFB, and IE treatments were inoculated with 30 g/hL of Lalvin EC-1118 yeast (Lallemand, Montreal, Quebec, Canada). Lachancea treatments were inoculated with 35 g/hL of *Lachancea thermotolerans* (Laktia, Lallemand, Montreal, Quebec, Canada) and were not subsequently inoculated with a strain of *Saccharomyces cerevisiae*.

Cap management consisted of two 2-minute punchdowns a day using a punch down tool (Vintner Vault, Paso Robles, CA, USA). The average fermentation temperature for Nebbiolo and Tannat treatments during alcoholic fermentation was 21 °C. For Nebbiolo, on Day 3 of alcoholic fermentation (AF), nutrients were added to all tanks in the form of 50 g/hL Fermaid K (Lallemand, Montreal, Quebec, Canada), and on Day 5, all tanks except for MLFB were coinoculated with malolactic bacteria (Lactoenos™ B7 *Direct*, Laffort, Bordeaux, France). For Tannat, on Day 2 of AF, nutrients were added in the form of 65 g/hL Fermaid K (Lallemand, Montreal, Quebec, Canada), and on Day 4, all tanks except for MLFB were coinoculated with malolactic bacteria (Lalvin VP41™, Lallemand, Montreal, Quebec, Canada). Wines were pressed from tanks into kegs on Day 11, after which AF was complete. AF was considered complete when wines reached < 2 g/L of residual sugar, and MLF was considered complete when wines achieved < 0.2 g/L of malic acid. Following AF,

MLFB wines were adjusted to 0.6 molecular SO_2 and stored in a cold room. After completion of MLF, IE wines were put through an ion exchange column (FreeK$^+$ column, Agrovin, Ciudad Real, Spain) and blended back at a 30 % proportion. This blending ratio was selected based on preliminary bench trials conducted on Nebbiolo wines investigating blending with 20 %, 25 %, and 30 % treated wine. It was decided that a 30 % IE blend achieved the greatest reduction in pH while maintaining positive sensory characteristics. The same blending ratio was applied to both Nebbiolo and Tannat wines to ensure consistency. Control, Lachancea, and IE wines were then adjusted to 0.35 molecular SO_2 and moved into a cold room for a 4-week period of tartrate stabilisation. All wines were then filtered through a plate and frame filter (Super Jet, Buon Vino, Cambridge, Ontario, Canada) using 14, 7, and 2 µm cellulose filter pads (Vintner›s Vault, Paso Robles, CA, USA), readjusted to 0.35 molecular SO_2, and bottled.

2. Chemical analysis

2.1 Monitoring Brix and oxidation-reduction potential during alcoholic fermentation

Total soluble solids (Brix) were measured using a temperature-compensated densimeter (DMA, Anton Paar, Graz, Austria). Oxidation-reduction potential (ORP) was measured during alcoholic fermentation of Control and Lachancea treatments using a platinum electrode with a silver/silver chloride (Ag/AgCl) reference electrode (EasyFerm Plus ORP Arc 120, Hamilton, Reno, Nevada, USA). Probes were housed in stainless-steel enclosures (Beringer Vineyards, Saint Helena, CA, USA) to avoid liquid and solids from the must interfering with the reading and were placed 38 cm above the bottom of the tank and slightly off centre to avoid interfering with cap management protocol (Parnigoni *et al*., 2025). ORP probes were connected to redox control boxes (MeshVines, Davis, CA, USA) and measured the ORP of the must every minute.

2.2 Basic chemistry of the wines

Basic chemistry and cations were measured at bottling. Ethanol content (% *v/v*) was measured by near-infrared spectroscopy using an Alcolyzer Wine M analysis system (Anton Paar, Graz, Austria). Titratable acidity (TA) was measured using a Wine Titrator (Hanna Instruments, Woonsocket, RI, USA) and pH was measured using a pH probe (Thermo Fisher Scientific, Waltham, MA, USA). Organic acids, including malic acid, lactic acid, and acetic acid, as well as cations, including iron, copper, potassium, and calcium, were measured enzymatically (Spica, Admeo, Angwin, CA, USA) using commercially available kits (Biosystems, Barcelona, Spain).

2.3 Accelerated ageing

Accelerated ageing was executed by bottling the wines in 50 mL air-tight glass ampules (Wheaton Industries, Millville, NJ, USA), followed by incubation for 5 weeks at 38 °C (Heratherm 120 V incubator, Thermo Fisher Scientific, Waltham, MA, USA) to mimic approximately 2 years of bottle ageing, as previously described (Casassa *et al*., 2023).

2.4 Spectrophotometric analysis

Colour, anthocyanins, tannins, polymeric pigments, and total phenolics were measured throughout winemaking and ageing at Day 6 of alcoholic fermentation, pressing, bottling, 3 months of bottle ageing, 6 months of bottle ageing, and following accelerated ageing. A Cary 60 UV-Vis spectrophotometer with an 18-position autosampler (Agilent Technologies, Santa Clara, CA, USA) was used for all analyses. Colour was determined from spectral scans (200 to 800 nm) collected in 1 mm quartz cuvettes using Cary WinUV Colour software (Version 6.0, Startek Technology, Denver, CO, USA), with total colour calculated as the sum of absorbances at 420, 520, and 620 nm. Anthocyanins, tannins, polymeric pigments, and total phenolics were quantified using the Adams-Harbertson assay (Harbertson *et al*., 2003). Anthocyanins were expressed as mg/L malvidin-3-glucoside equivalents, tannins were measured by protein precipitation and expressed as (+)-catechin equivalents (CE), polymeric pigments were quantified according to the Adams-Harbertson assay, and total phenolic concentrations were expressed as mg/L catechin equivalents.

2.5 Anthocyanins, anthocyanin-derived pigments, and flavonols

Anthocyanins and anthocyanin-derived pigments were measured at bottling and following accelerated ageing, while flavonols were measured at 3 months of bottle ageing and following accelerated ageing using high-performance liquid chromatography with diode array detection (HPLC-DAD). Analyses were conducted using an Agilent 1100 series HPLC system with a diode array detector (Agilent Technologies, Santa Clara, CA). Chromatographic separation was performed on a reversed-phase C18 column (Agilent Zorbax Eclipse Plus, 4.6 × 100 mm, 3.5 µm particle size) with a corresponding guard column, both maintained at 30 °C. Separation was achieved using a gradient of two solvents: A) 5 % formic acid in water and B) methanol, delivered at a flow rate of 1 mL/min. The gradient program progressed from 23 % B at 0 min to 26 % B at 5 min, 60 % B at 15 min, and 100 % B at 16 min with a stop time of 19 minutes. The column was equilibrated for 2 minutes at 23 % B before injection. Sample injections were 20 µL. Spectral data were collected between 190 and 700 nm, with quantification of anthocyanins and flavonols based on absorbance peaks at 520 nm and 356 nm, respectively. All solvents used were HPLC grade (Thermo Fisher Scientific, Waltham, MA, USA).

Monomeric anthocyanins were quantified using a malvidin-3-glucoside chloride standard (Extrasynthèse, Lyon, FRA) with a calibration curve with $R^2 = 0.99$. Anthocyanin-derived compounds were categorized into six groups: (1) malvidin-3-glucoside, (2) petunidin-3-glucoside, (3) peonidin-3-glucoside, (4) delphinidin-3-glucoside, (5) cyanidin-3-glucoside, (6) acylated forms such as acetylated and coumaroylated monoglucosides, and (7) polymeric pigments and vitisins including Vitisin A and B. Flavonol concentrations were quantified using a quercetin-3-glucoside reference standard (Sigma-Aldrich, St. Louis, MO, USA) with a calibration curve with $R^2 = 0.99$. Flavonols were grouped into two categories:

(1) quercetin derivatives including quercetin-3-glucoside, quercetin-3-glucuronide, and quercetin aglycone, and (2) non-quercetin derivatives including myricetin, laricitrin, isorhamnetin, syringetin, kaempferol, and their respective derivatives. Anthocyanins, anthocyanin-derived pigments, and flavonols were identified using known elution order from previous literature and using the maximum absorbance wavelength of each peak.

2.6 Monomeric flavan-3-ols

Wine samples were analysed at 3 months of bottle ageing and following accelerated ageing using an Agilent 1260 Infinity II HPLC system coupled to an Agilent Ultivo mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was carried out on a Zorbax Eclipse Plus C18 column (4.6 × 100 mm, 3.5 µm particle size; Agilent Technologies) with a 10 µL injection volume. The mobile phase consisted of A) 0.1 % formic acid in water and B) 0.1 % formic acid in acetonitrile, delivered at a flow rate of 0.8 mL/min, while the column temperature was maintained at 25 °C. A gradient program was applied: 3 % B at 0 and 2 min, 6 % B at 10 min, 42 % B at 25 min, 100 % B at 30 min, followed by 3 % B at 32.5 min. Samples were stored in an autosampler at 10 °C prior to injection.

Mass spectrometric detection was performed using electrospray ionisation in negative mode with a dual ESI source. Operating parameters included a drying gas flow of 11.0 L/min at 325 °C, nebuliser pressure of 35 psi, capillary voltage of 3000 V, and a fragmentor voltage of 130 V. Data were acquired in full-scan mode over an *m/z* range of 100–1400. The instrument was calibrated before each analytical sequence following standard manufacturer procedures.

2.7 Volatile compounds

Volatile compounds were measured at 3 months of bottle ageing since this was the time point conducted closest to sensory analysis. Volatile compounds were extracted from wine samples using stir bar sorptive extraction (SBSE) with polydimethylsiloxane (PDMS)-coated stir bars (Twister®, Gerstel, Germany) according to a previous method (Marín-San Román *et al*., 2022). Stir bars were placed directly into the samples and agitated at 1000 rpm for 1 hour at room temperature. Following extraction, analytes were thermally desorbed using a Gerstel thermal desorption unit (TDU2) coupled with a cooled injection system (CIS-4) equipped with a liner containing 20 mg of Tenax TA®. Desorption and gas chromatography conditions were consistent with those previously reported (Marín-San Román *et al*., 2022).

The resulting volatile compounds were separated using an Agilent 8890 gas chromatograph interfaced with an Agilent 7000D triple quadrupole mass spectrometer (Agilent Technologies), operated in single quadrupole mode. Quantification was achieved using calibration curves prepared from authentic standards in a synthetic wine (13.5 % ethanol, 5 g/L tartaric acid, pH 3.6, 20 mg/L SO_2). Standards (purity > 95 %) included ethyl butyrate, ethyl isovalerate, isoamyl acetate, ethyl hexanoate, hexyl acetate, ethyl *n*-octanoate, ethyl decanoate, methyl salicylate, 2-phenylethyl acetate, ethyl cinnamate, geraniol, *cis*-rose oxide, linalool, β-citronellol, nerol, β-ionone, and *trans*-nerolidol.

3. Sensory analysis

3.1 Modified Pivot© Profile

Modified Pivot© Profile sensory analysis was conducted on Nebbiolo wines at 3 months of bottle ageing using RedJade sensory software (RedJade, Pleasant Hill, CA, USA), following the methodology described by Stoffel *et al*. (2025) and Pearson *et al*. (2020). The Modified Pivot© Profile conducted in this study used a list of sensory attributes that was predetermined by a group of seven experienced tasters prior to training and formal evaluations. The control wines were designated as the Pivot© and used as the reference for all comparisons. For each attribute, panellists indicated whether the sample was perceived as "more than the Pivot©" or "less than the Pivot©", which were coded as 1 and 0, respectively.

Attributes and standard references were selected prior to the panel by a group of seven experienced tasters. They included purple hue, colour saturation, bitterness, astringency, and acidity. The panel consisted of 10 wine professionals. Panellists completed a 2-h training session in which they were familiarised with sensory attributes, reference standards (Table S1), and evaluation procedures.

Formal evaluations were conducted in individual sensory booths under controlled lighting conditions. Colour attributes (purple hue and saturation) were assessed under daylight lighting (Luna 3, 26:26W, Zaniboni Lighting, Clearwater, FL, USA), while taste and mouthfeel attributes (bitterness, astringency, and acidity) were evaluated under red lighting (Luna 3AO, 18:18W, Zaniboni Lighting, Clearwater, FL, USA). Samples (30 mL) were served in clear, ISO-standard wine glasses labelled with randomised three-digit codes. Each evaluation consisted of paired samples, including one Pivot© and one treatment wine. Panellists evaluated each treatment, excluding the control, in triplicate. Panellists were instructed to expectorate all samples and to cleanse their palate between samples using water (Crystal Geyser, Olancha, CA, USA) and unsalted crackers (Nabisco, East Hanover, NJ, USA). The project was approved by the California Polytechnic State University Institutional Review Board (IRRB Protocol # 2020-058).

3.2 Time-intensity descriptive analysis

Time-intensity sensory analysis was conducted on Nebbiolo wines at 3 months of bottle ageing to evaluate the intensity of red fruit flavour on the palate over time. Evaluations were performed using RedJade sensory software (RedJade, Pleasant Hill, CA, USA) for both training and data collection. This project was approved by the California Polytechnic State University Institutional Review Board (IRRB Protocol # 2020-058).

The panel consisted of 11 wine professionals. Panellists completed a 2-h training session in which they were familiarised with the concept of time-intensity evaluation, RedJade software, and the sensory attribute of red fruit

flavour. Training included exposure to low- and high-intensity red fruit reference standards, as well as all treatment wines (Table S2). Panellists initially practised evaluating samples using paper ballots before transitioning to practising using RedJade in the sensory booths.

During formal evaluation, panellists were instructed to take the entire sample (15 mL), swish for 5 seconds using a built-in timer, expectorate, and immediately begin recording perceived intensity. Intensity of red fruit flavour was recorded continuously over a 45-second period using a horizontal scale ranging from 0 (no perceived intensity) to 10 (maximum perceived intensity). Panellists adjusted the scale to reflect perceived changes in intensity and returned the scale to zero once the sensation was no longer perceived. Evaluations were conducted in individual sensory booths. Samples were served in clear, ISO-standard wine glasses labelled with randomised three-digit codes. Each wine treatment was evaluated in triplicate, with presentation order randomised across panellists. Panellists were instructed to cleanse their palate between samples using water (Crystal Geyser, Olancha, CA, USA) and unsalted crackers (Nabisco, East Hanover, NJ, USA). Time-intensity parameters, including area under the curve (AUC) and maximum intensity (I_max), were calculated from the recorded data for each sample.

4. Data analysis

Data were analysed using XLSTAT software (Version 2025.2.0, Paris, France). A one-way analysis of variance (ANOVA) and two-way ANOVA and Fisher's LSD comparison tests ($p < 0.05$) were conducted on the basic chemical composition, phenolic composition, and volatile chemistry of the different treatments within each varietal. Plots and graphs were produced using Graphpad Prism software 11.0.0 (Graphpad, La Jolla, CA, USA). A one-way ANOVA was conducted on time-intensity parameters including area under the curve (AUC) and maximum intensity (I_max).

RESULTS AND DISCUSSION

1. Monitoring of alcoholic fermentation, malolactic fermentation, and oxidation-reduction potential

Analysis of fermentation kinetics (Figure 1) showed that musts inoculated with *Lachancea thermotolerans* progressed more slowly than the other treatments inoculated with *Saccharomyces cerevisiae*. The latter trend agrees with previous studies showing that *Lachancea thermotolerans* has a lower fermentative power compared to *Saccharomyces cerevisiae* (Vicente *et al.*, 2024). This was evident in both Nebbiolo and Tannat, where Lachancea treatments began to fall behind around mid-ferment (Days 2 to 6) for both varietals, but it should be noted that Lachancea wines were not co-inoculated with a *Saccharomyces cerevisiae* strain. While Lachancea ferments were indeed slightly more sluggish than all other treatments, they were able to complete AF within the same time frame of 10 days, achieving dryness (< 2 g/L residual sugar). For both varietals, Lachancea treatments also took longer to complete malolactic fermentation (MLF) compared to control treatments (Table S3). Lachancea wines took 1.7 and 2.4 times longer than control wines to complete MLF for Nebbiolo and Tannat wines, respectively.

Oxidation-reduction potential (ORP) was monitored during alcoholic fermentation to uncover potential yeast-related effects (*i.e.*, *Saccharomyces cerevisiae vs Lachancea thermotolerans*) on ORP. ORP decreased rapidly in both treatments and varietals following inoculation (Figure 2), reflecting the increase in yeast populations creating a reduced environment through oxygen consumption during fermentation (Salmon, 2006). The spikes observed in Figure 2 are attributed to, and coincide with, daily cap management operations, which consisted of two punch-downs per day, which temporarily increased oxygen and ORP. In both Nebbiolo and Tannat, Lachancea

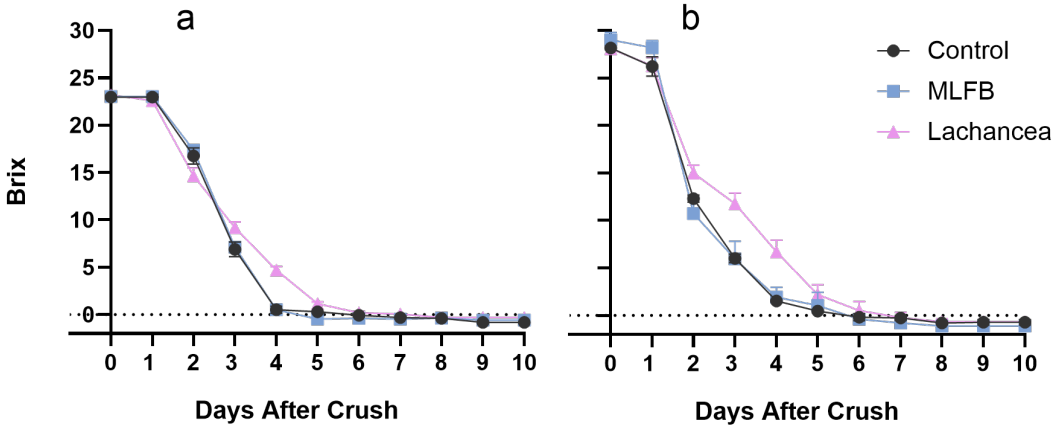

FIGURE 1. Evolution of sugar consumption measured in Brix during alcoholic fermentation of a) Nebbiolo and b) Tannat wines. Each data point represents the mean of three replicates ($n = 3$), and error bars represent the standard error of the mean. The horizontal dotted line indicates 0 Brix.

treatment maintained lower ORP values compared to the control (*i.e.*, *Saccharomyces cerevisiae*), indicating a more reduced environment throughout fermentation. Accordingly, Lachancea wines exhibited a greater proportion of time under reductive conditions across both varietals, with a higher negative area under the curve (NAUC) and a lower positive area under the curve (PAUC). In contrast, the control treatment for both varietals showed a higher PAUC and lower NAUC, indicating more time spent under more oxidative conditions. These differences should be interpreted cautiously due to the substantially however different fermentation kinetics observed between control and Lachancea treatments (Figure 1). Differences in ORP may be attributed to variations in metabolic activity between *Lachancea thermotolerans* and *Saccharomyces cerevisiae* fermentations, as previously reported for non-*Saccharomyces* yeasts (Escott *et al*., 2018; Sánchez-Suárez *et al*., 2026; Vicente *et al*., 2024). Previous studies have also found that *Lachancea thermotolerans* reportedly produces 2 to 5 times more glutathione than *Saccharomyces cerevisiae*, which is an antioxidant (Vicente *et al*., 2021), and, as a thiol, has been shown to exert lowering effects on ORP (Wright *et al*., 2026). However, glutathione was not measured in this study, and further studies directly quantifying the concentrations of glutathione associated with control and Lachancea treatments would be required to determine its contribution to the observed differences in ORP. Lachancea treatment also displayed more fluctuation in ORP during punch-downs, whereas the control treatment maintained ORP values with less variability (Figure 2).

2. Basic chemistry of the wines

2.1 pH, titratable acidity, and ethanol

Table 1 shows a detailed overview of the basic chemical composition of Nebbiolo and Tannat wines. In both varietals, the acid management strategy significantly influenced pH, titratable acidity (TA), and ethanol concentration at bottling. IE wines consistently exhibited the lowest pH values, followed by MLFB and Lachancea treatments, while control wines maintained the highest pH values. In Nebbiolo, pH decreased by 0.28 units in IE wines relative to the control (3.52 *vs* 3.80), while in Tannat, IE and Lachancea treatments resulted in the lowest pH values (3.54 and 3.60, respectively) compared to the control (3.87). The most effective strategy for reducing pH was ion exchange. This is explained by its ability to remove cations, mainly potassium, from the wine, replacing them with a hydrogen ion (H^+) (Ponce *et al*., 2018). The ability of H^+ from acids to dissociate directly correlates to pH, which explains why IE wines exhibited lower pH values but only moderate increases in TA relative to other treatments.

Lachancea treatments produced the highest TA across both varietals, reflecting the yeast's ability to convert sugars into lactic acid during AF (Table 1). While Lachancea wines substantially increased TA (1.4 g/L higher than the control), this treatment had less of an effect on pH, only decreasing it by 0.16 units. This can be explained by differences in acid dissociation behaviour. Lactic acid has a higher pKa (3.86) than comparatively stronger organic acids including tartaric (pKa_1 = 2.98, pKa_2 = 4.34), meaning that increases in lactic acid contribute more to TA than to free hydrogen ion concentration and, consequently, pH (Payan *et al*., 2023).

In Nebbiolo, MLFB wines showed higher ethanol levels compared to Lachancea and IE wines (0.46 and 0.44 % *v/v* more, respectively). In Tannat, MLFB resulted in wines with higher ethanol compared to all other treatments (0.89 % *v/v* more than the control). This is likely the result of all other treatments being co-inoculated with malolactic bacteria during AF. As a result of co-inoculation with malolactic bacteria during alcoholic fermentation, when sugar is still available for lactic acid bacteria to consume, a diversion of this sugar to metabolic by-products other than ethanol, including D-lactate and acetate, can occur, as previously observed (Jussier *et al*., 2006; Minnaar *et al*., 2019; Contreras *et al*., 2018).

Additionally, the relationship between observed and predicted values for the IE blended wines at a 30 % rate for selected parameters was calculated (Figure S1). Predicted values for pH, TA, and ethanol were calculated as the statistical mean of that variable, considering a blending ratio of 30:70 as applied to the IE wines ([0.30 × {IE treated wine} + 0.70 × {Control}]). The linear model was based on volume-wise proportioning, assuming no interactive effects. Percentage deviation from additive effects was assumed as (observed value – predicted value)/predicted value) × 100. Supra-additive effects were considered as a > 5 % deviation above linear averaging effects, sub-additive effects a deviation of < 5 % below linear averaging effects, and additive effects were established as a deviation within ± 5 %. Ethanol and TA showed additive effects for both varietal wines, suggesting that upon ion exchange, these two parameters averaged linearly. The practical implication of the latter is that, for ethanol and titratable acidity, these parameters can be predicted before blending with pure IE wines. Predicted pH values on 30 % IE blended wines were about 3.30 for both varietals, even though observed values were between pH 3.50 and 3.55 (Figure S1). Therefore, pH showed supra-additive effects for both varietals, indicating that even though IE effectively decreased pH in the blended wines (Table 1), it did so less than proportionally when considering the pH of the pure IE wine.

2.2 Organic acid composition

As intended, the acid adjustment treatments also affected organic acid composition of the finished wines (Table 1). Lachancea produced wines with the highest concentrations of lactic acid in both Nebbiolo and Tannat wines, consistent with the known ability of *Lachancea thermotolerans* to produce lactic acid from sugars during AF. While Lachancea wines did have more lactic acid, the Laktia strain herein employed only produced 0.83 and 0.50 g/L of lactic acid more compared to the control in Nebbiolo and Tannat wines, respectively. Since lactic acid bacteria were co-inoculated during alcoholic fermentation, this increase in the final

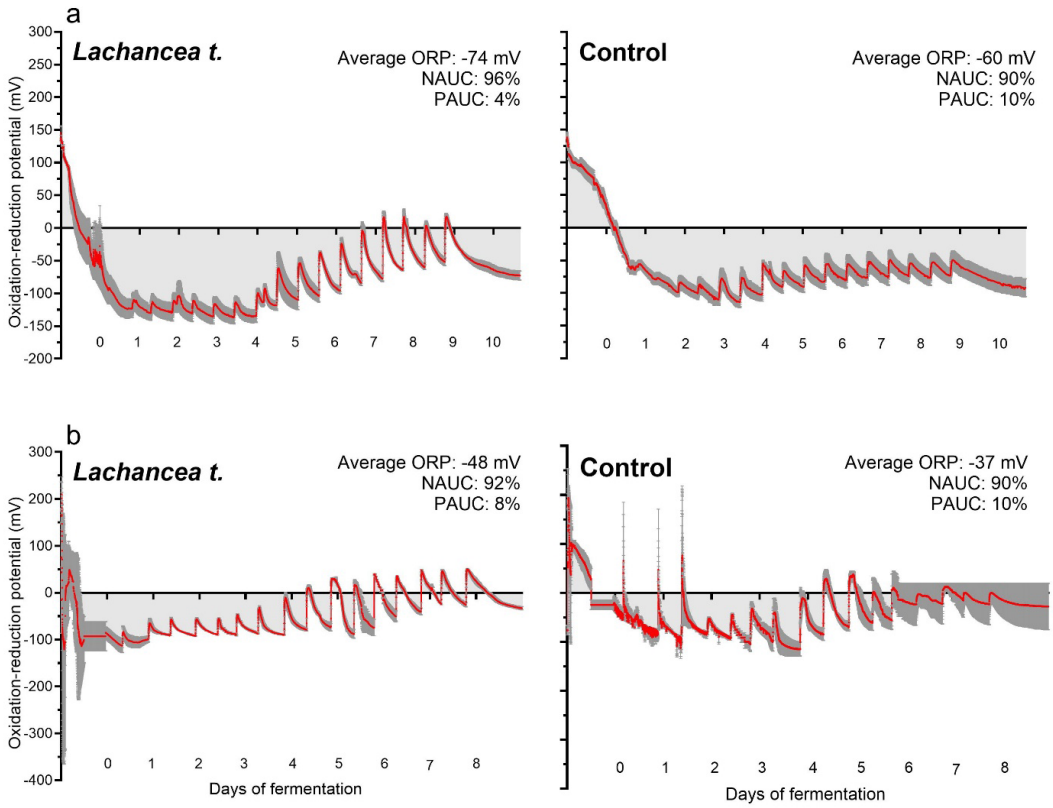

FIGURE 2. Oxidation-reduction potential of Lachancea and control treatments during alcoholic fermentation for a) Nebbiolo and b) Tannat wines. Dark grey represents the standard error of the mean (n = 3). Negative area under the curve (NAUC) represents the proportion of cumulative area where ORP values were below 0 mV, and positive area under the curve (PAUC) represents the proportion of cumulative area where ORP values were above 0 mV.

TABLE 1. Basic chemical composition of Nebbiolo and Tannat wines.

Varietal	Treatment	Ethanol (% *v/v*)	Titratable acidity (g/L tartaric acid)	pH	Malic acid (g/L)	Lactic acid (g/L)	Acetic acid (g/L)
Nebbiolo	Control	13.1 ab	5.65 c	3.80 a	0.01 b	1.55 b	0.35 b
	MLFB	13.3 a	6.80 ab	3.68 b	2.14 a	0.30 c	0.08 d
	Lachancea	12.8 b	7.05 a	3.75 ab	0.01 b	2.38 a	0.63 a
	IE	12.8 b	6.57 b	3.52 c	0.15 b	1.42 b	0.28 c
	p-value	**0.008**	**0.000**	**0.000**	**<0.0001**	**<0.0001**	**<0.0001**
Tannat	Control	15.4 b	5.88 c	3.87 a	0.02 b	1.08 b	0.42 b
	MLFB	16.3 a	6.82 b	3.68 b	1.27 a	0.09 c	0.19 c
	Lachancea	15.6 b	7.28 a	3.60 c	0.03 b	1.58 a	0.47 a
	IE	15.3 b	6.67 b	3.54 c	0.02 b	1.14 b	0.40 b
	p-value	**0.001**	**0.000**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Two-Way ANOVA							
Treatment effect	Control	14.2 b	5.77 c	3.84 a	0.01 b	1.31 b	0.39 b
	MLFB	14.8 a	6.81 b	3.68 b	1.70 a	0.19 c	0.14 d
	Lachancea	14.2 b	7.17 a	3.68 b	0.02 b	1.98 a	0.55 a
	IE	14.1 b	6.62 b	3.53 c	0.09 b	1.28 b	0.34 c
	p-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Varietal effect	Nebbiolo	13.0 b	6.52 a	3.69 a	0.58 a	1.41 a	0.34 b
	Tannat	15.7 a	6.66 a	3.67 a	0.33 b	0.97 b	0.37 a
	p-value	**<0.0001**	0.094	0.373	**0.001**	**<0.0001**	**0.010**

* Bold values indicate significant differences between treatments for the Fisher (LSD) test and $p < 0.05$.

lactic acid concentrations reflects contributions from both *Lachancea thermotolerans* and malolactic fermentation. Because of this experimental design, the proportion of the final lactic acid originating from *Lachancea thermotolerans* metabolism *versus* MLF cannot be determined. Previous studies using the Laktia strain of *Lachancea thermotolerans* have also found it to be a low producer of lactic acid compared to other strains, resulting in increases ranging from 0.95 to 1.19 g/L lactic acid compared to control wines (Sánchez-Suárez *et al*., 2026; Vaquero *et al*., 2021; Vicente *et al*., 2024). Different strains of *Lachancea thermotolerans* produce varying concentrations of lactic acid due to differences in the expression of the lactate dehydrogenase (LDH) gene (Gatto *et al*., 2020; Sgouros *et al*., 2020). In contrast, MLFB wines had the lowest lactic acid concentrations (1.12 g/L less than control wines). Malic acid concentrations were also highest in MLFB wines across both varietals (1.69 g/L more than control wines), confirming successful MLF inhibition and retention of malic acid. Lachancea wines also produced the highest concentrations of acetic acid at around 0.55 g/L. This may be attributed to slower alcoholic fermentation kinetics in Lachancea wines (Figure 1), which may have influenced acetic acid accumulation. However, these values remained well below the acetic acid odour detection threshold (Macías *et al*., 2013).

2.3 Cations

The acid adjustment treatments had significant effects across both varietals for cations including iron, copper, potassium, and calcium in finished wines (Table 2). In both Nebbiolo and Tannat, Lachancea resulted in wines with the lowest copper concentrations (2.91 and 0.74 ppm less than the control, respectively). A possible explanation for the latter result is that the yeast cell walls of *Lachancea thermotolerans* absorb copper, lowering the concentrations in the finished wine. Copper catalyses oxidation reactions in wine; therefore, reduced copper concentrations may improve oxidative stability (Danilewicz, 2003). Potassium concentrations did not differ in Nebbiolo wines. However, in Tannat wines, all treatments resulted in lower potassium concentrations compared to the control. A two-way ANOVA revealed that IE treatment was the most effective at decreasing potassium concentrations (amounting to a 9 % reduction relative to the control). This is consistent with previous research that found lower potassium levels in wines passed through ion exchange columns (Cisilotto *et al*., 2020). Calcium seemed to vary the most between treatments and was the most consistent between varietals, showing that IE wines had lower concentrations compared to the control, while MLFB and Lachancea wines had higher concentrations. Previous studies have suggested that ion exchange is effective at removing calcium, especially since cationic resins preferentially exchange H^+ with cations of higher valence (Cisilotto *et al*., 2020). Therefore, calcium (Ca^{2+}) will be more readily exchanged compared to potassium (K^+). Decreased potassium and calcium concentrations will improve tartrate stability in the finished wines by decreasing the availability of these cations to form potassium bitartrate and calcium tartrate crystals.

TABLE 2. Cation concentrations of Nebbiolo and Tannat wines.

Varietal	Treatment	Iron (ppm)	Copper (ppm)	Potassium (ppm)	Calcium (ppm)
Nebbiolo	Control	1.7 b	3.8 a	826 a	57.3 b
	MLFB	1.3 c	4.6 a	885 a	67.0 a
	Lachancea	1.9 a	0.9 b	908 a	71.3 a
	IE	1.3 d	4.8 a	834 a	49.0 c
	p-value	**<0.0001**	**<0.0001**	0.157	**<0.0001**
Tannat	Control	2.2 ab	1.3 b	1122 a	51.3 b
	MLFB	2.3 ab	1.9 a	993 b	64.7 a
	Lachancea	2.3 a	0.5 c	929 c	64.7 a
	IE	2.2 b	1.6 ab	932 bc	45.0 c
	p-value	0.201	**0.000**	**0.000**	**<0.0001**
Two-Way ANOVA					
Treatment effect	Control	2.0 b	2.5 b	974 a	54.3 b
	MLFB	1.8 c	3.3 a	939 ab	65.8 a
	Lachancea	2.1 a	0.7 c	918 bc	68.0 a
	IE	1.7 d	3.2 a	883 c	47.0 c
	p-value	**<0.0001**	**<0.0001**	**0.009**	**<0.0001**
Varietal effect	Nebbiolo	1.5 b	3.6 a	863 b	61.2 a
	Tannat	2.2 a	1.3 b	994 a	56.4 b
	p-value	**<0.0001**	**<0.0001**	**0.009**	**<0.0001**

* Bold values indicate significant differences between treatments for the Fisher (LSD) test and $p < 0.05$.

3. Colour and phenolic composition of the wines

3.1 Wine colour and colour stability

The different acid management strategies significantly influenced total colour in both Nebbiolo and Tannat wines (Figure 3), with visual differences being apparent in Nebbiolo wines after 1 year of bottle ageing (Figure S2). At bottling, MLFB and IE wines had significantly higher colour than control wines in both varietals. A two-way ANOVA indicated a significant varietal × treatment interaction at bottling, highlighting how the different phenolic compositions between varieties affected the treatment response. The enhanced colour observed in both Nebbiolo and Tannat MLFB and IE wines is consistent with their lower pH values, which favour the stability of flavylium cation forms of anthocyanins and promote anthocyanin-tannin condensation reactions (Forino *et al*., 2020; Gambuti *et al*., 2022). These reactions contribute to the formation of polymeric pigments, which are more resistant to SO_2 bleaching and oxidative degradation during ageing (Casassa & Harbertson, 2014).

After accelerated ageing, all Nebbiolo wine treatments had higher total colour than the control, suggesting that lowering the pH promoted pigment stabilisation across treatments. This was also observed after 1 year of traditional bottle ageing (Figure S2). In Tannat wines, only MLFB and IE wines retained significantly higher total colour than the control and Lachancea wines, but these differences were less apparent, seemingly due to the extremely naturally high colour saturation of Tannat wines (Figure S2). These findings suggest that acid management strategies that contribute more to pH while retaining tannins, including MLFB and IE treatments, play an important role in preserving colour during wine ageing, especially in varietals with more colour such as Tannat.

3.2 Phenolic composition

Phenolic composition at bottling and after accelerated ageing was strongly affected by treatment, with significant varietal × treatment interactions for anthocyanins, total polymeric pigments (TPP), tannins, and total phenolics (Table S4). At bottling, MLFB wines had 16 % more polymeric pigments relative to control wines, while Lachancea wines consistently showed the lowest polymeric pigment content (13 % less than the control). MLFB and IE wines had higher total tannin (19 % more) and total phenolic concentrations (5 % and 11 % more, respectively) than control wines, while Lachancea wines had the lowest concentrations (28 % and 23 % less, respectively).

Higher tannin and polymeric pigment concentrations in MLFB wines suggest enhanced potential for colour stability during ageing in these wines, especially in the case of Nebbiolo wines, as observed after 1 year of bottle ageing (Figure S2). MLFB wines retained malic acid, maintaining a lower pH compared to control wines. Lower pH favours conditions for anthocyanin-tannin interactions and, overall, polymeric pigment formation (Gambuti *et al*., 2022). Wines that undergo MLF are also more likely to have lower acetaldehyde and pyruvic acid concentrations since *Oenococcus oeni* degrades these compounds during MLF (Jackowetz & Mira De Orduña, 2012). At bottling, MLFB wines had 26 and 21 mg/L higher acetaldehyde concentrations than control wines in Nebbiolo and Tannat wines, respectively. Crucially, acetaldehyde and pyruvic acid are both mediators for polymeric pigment formation (Morata *et al*., 2007). Therefore, blocking MLF helped to increase polymeric pigments in the finished wines since these compounds were more available to react and produce polymeric pigments. IE wines also had more tannin and phenolic concentrations compared to the control (Table S4), further supporting the role that a comparatively lower pH may play in facilitating phenolic stabilisation. As previously alluded, visible differences in colour in the Tannat wines were less pronounced (Figure S2), likely due to the higher concentrations of anthocyanins and polymeric pigments in Tannat wines compared to Nebbiolo wines (Figures 4 and 5), as well as their higher total colour (Figure 3), approximately five-fold higher relative to that of Nebbiolo wines. Therefore, the impact of treatments on colour in Tannat wines was less impactful, likely due to the higher initial anthocyanin and phenolic composition of Tannat relative to Nebbiolo.

Lachancea wines consistently exhibited lower tannin, polymeric pigment, and total phenolic concentrations across both varietals and time points (Figures 4 and 5). Although Lachancea wines produced higher concentrations of anthocyanins at bottling in Nebbiolo wines, these pigments were less effectively stabilised over time, likely due to reduced tannin availability for polymerisation.

4. Anthocyanins, anthocyanin-derived pigments, and flavonols

4.1 Anthocyanins and anthocyanin-derived pigments

Figure 6 shows the evolution of anthocyanins and anthocyanin-derived pigments throughout winemaking and ageing for Nebbiolo and Tannat wines. All three treatments significantly influenced the concentration of anthocyanins in Nebbiolo wines at bottling and accelerated ageing (Table S5). In Nebbiolo, Lachancea wines had the highest concentration of several anthocyanins, including malvidin-3-glucoside, peonidin-3-glucoside, and cyanidin-3-glucoside, compared to all other treatments (22 %, 106 %, and 163 % higher than the control, respectively), while MLFB wines had significantly higher concentrations of petunidin-3-glucoside relative to the control and Lachancea treatments (32 % and 39 % higher, respectively).

Following accelerated ageing in Nebbiolo wines, Lachancea wines retained significantly higher concentrations of peonidin-3-glucoside, delphinidin-3-glucoside, and cyanidin-3-glucoside compared to the other treatments (93 %, 25 %, and 114 % higher than the control, respectively). Concentrations of malvidin-3-glucoside after accelerated ageing were highest in both control and Lachancea wines (14.6 mg/L for both), while MLFB and IE wines showed lower levels (10.2 and 11.9 mg/L, respectively).

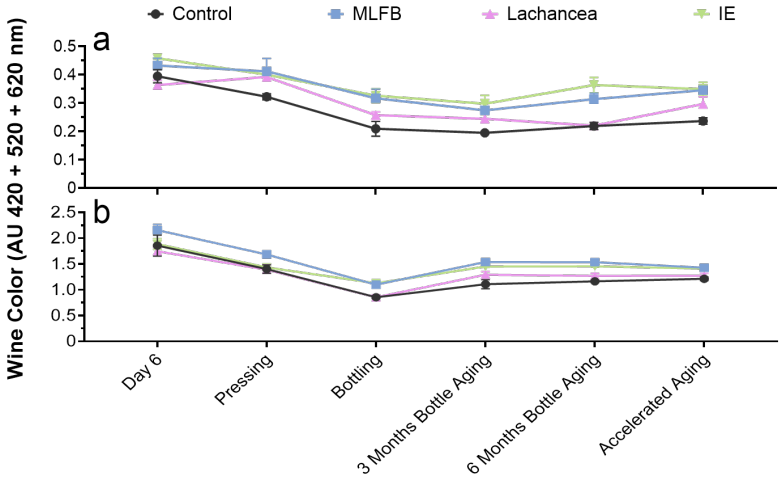

FIGURE 3. Evolution of total colour of a) Nebbiolo and b) Tannat wines throughout winemaking.

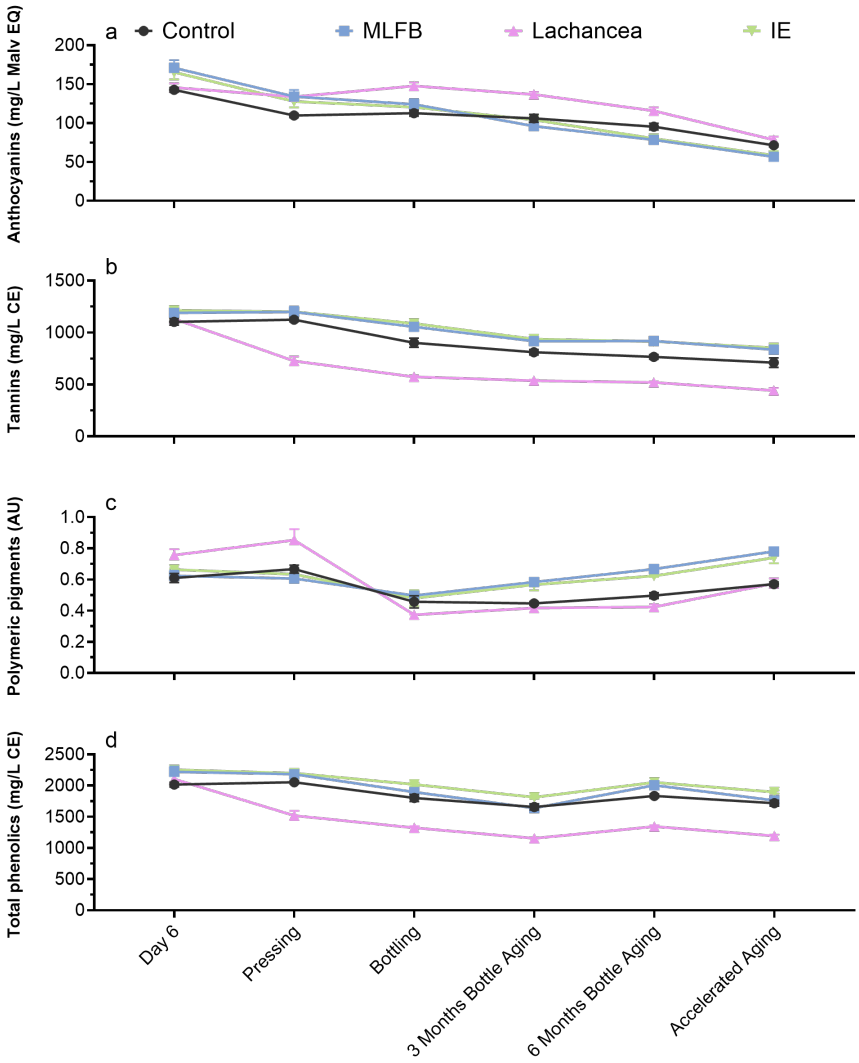

FIGURE 4. Phenolic composition of Nebbiolo wines, including a) anthocyanins, b) tannins, c) polymeric pigments, and d) total phenolics across winemaking and ageing. Data points represent average values between three replicates (n = 3) of each treatment, and bars represent the standard error of the mean.

These findings suggest that Lachancea fermentations promoted higher initial concentrations and greater retention of several monomeric anthocyanins during ageing in Nebbiolo wines. Previous studies have found that a lower pH during maceration favours the extraction of anthocyanins (Forino *et al.*, 2019), which could explain why Lachancea wines showed the highest anthocyanin concentrations compared to all other treatments. Because *Lachancea thermotolerans* produces lactic acid during alcoholic fermentation, acidification for Lachancea treatments occurred during maceration, creating a more favourable environment for anthocyanin extraction compared to MLFB and IE treatments where acidification occurred later. However, despite higher monomeric anthocyanin concentrations, Lachancea wines had lower levels of polymeric pigments in previous analyses, indicating reduced formation of these pigments into more stable polymeric forms.

Similarly, in Tannat wines, treatments also significantly influenced anthocyanin composition at bottling and accelerated ageing (Table S5). MLFB wines exhibited the highest concentrations of malvidin-3-glucoside, acylated anthocyanins, and polymeric pigments compared to all other treatments (21 %, 21 %, and 35 % higher than the control, respectively). Following accelerated ageing, control and MLFB wines maintained significantly higher levels of polymeric pigments (40.8 and 44.0 mg/L, respectively) compared to Lachancea and IE wines (32.2 and 35.4 mg/L, respectively). This is consistent with previous studies that have also found reduced concentrations of anthocyanins in wines that go through MLF (Izquierdo-Cañas *et al.*, 2016). These results indicate that MLFB in Tannat wines may promote higher initial anthocyanin concentrations and greater formation of polymeric pigments during ageing.

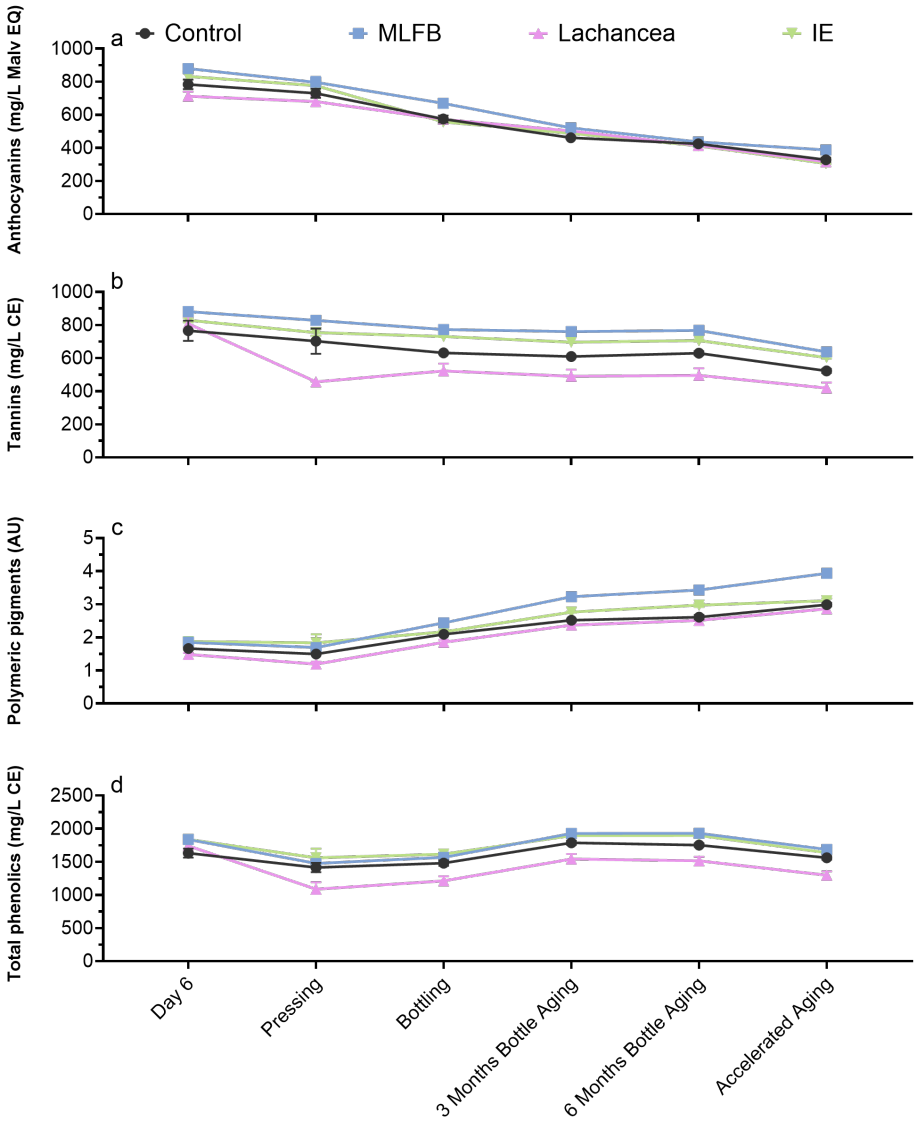

FIGURE 5. Phenolic composition of Tannat wines, including a) anthocyanins, b) tannins, c) polymeric pigments, and d) total phenolics across winemaking and ageing. Data points represent average values between three replicates (n = 3) of each treatment, and bars represent the standard error of the mean.

In addition to specific treatment effects, each varietal showed a distinct anthocyanin profile, especially from a quantitative standpoint. From a quantitative standpoint, the most prevalent monoglucosides in Nebbiolo wines were malvidin-3-glucoside and peonidin-3-glucoside, together contributing to approximately 80 to 85 % of the total anthocyanin makeup in finished wines. This is consistent with previous studies that have also found malvidin-3-glucoside and peonidin-3-glucoside to be the most prevalent monoglucosides in Nebbiolo wines (Guidoni *et al*., 2002). The most prevalent anthocyanins in Tannat wines were malvidin-3-glucoside and acylated anthocyanins, collectively contributing to approximately 86 to 88 % of the total anthocyanin concentration in finished wines, in agreement with previous reports on Tannat wines from Uruguay (González-Neves *et al*., 2012). Tannat wines also had significantly higher concentrations of total anthocyanins, about 6 to 8 times as much at bottling and throughout ageing (Figure 6). This is consistent with previous literature suggesting Tannat wines have higher phenolics and anthocyanin concentrations (González-Neves *et al*., 2004), while Nebbiolo wines have low anthocyanin concentrations (Casassa *et al*., 2022).

4.2 Flavonols

The evolution of flavonols at 3 months of bottle ageing and following accelerated ageing of Nebbiolo and Tannat wines is shown in Figure 7. In Nebbiolo wines at 3 months of bottle ageing and throughout accelerated ageing, there were no significant differences in flavonols between acid management strategies (Table S6). In Tannat wines, acid management strategy did show significant differences in flavonols at 3 months of bottle ageing and following accelerated ageing (Table S6). At 3 months of bottle ageing, MLFB and IE wines had higher quercetin (12 % and 11 % higher, respectively) and non-quercetin derivatives (18 % and 13 % higher, respectively) than control wines. Following accelerated ageing, MLFB wines had the highest quercetin and non-quercetin derivatives compared to all other treatments (18 % and 20 % higher than the control, respectively). IE wines had higher quercetin derivatives than the control and Lachancea wines (8 % and 9 % higher, respectively). These results suggest that blocking MLF seemed to produce wines with higher flavonol concentrations for Tannat, including after accelerated ageing. IE wines also seemed to preserve more flavonol concentrations compared to the control, but it was less effective than blocking MLF.

Nebbiolo wines also had a greater proportion of quercetin derivatives compared to non-quercetin derivatives, while Tannat wines showed the opposite, with a higher proportion of non-quercetin derivatives (Figure 7). The quercetin:non-quercetin ratio was approximately 10:3 for Nebbiolo and 1:2 for Tannat. A previous study also found that Nebbiolo grapes have a higher proportion of quercetin compared to other flavonols, with quercetin making up approximately 80 % of the total flavonol concentration for Nebbiolo grapes in a study covering two successive years (Ferrandino *et al*., 2012). A previous study on Tannat also reported a ratio of quercetin:non-quercetin derivatives of approximately 1:2 for Tannat wines, highlighting the dominance of non-quercetin derivatives including myriciten, laricetrin, syringetin, and their respective derivatives in Tannat (Boido *et al*., 2011).

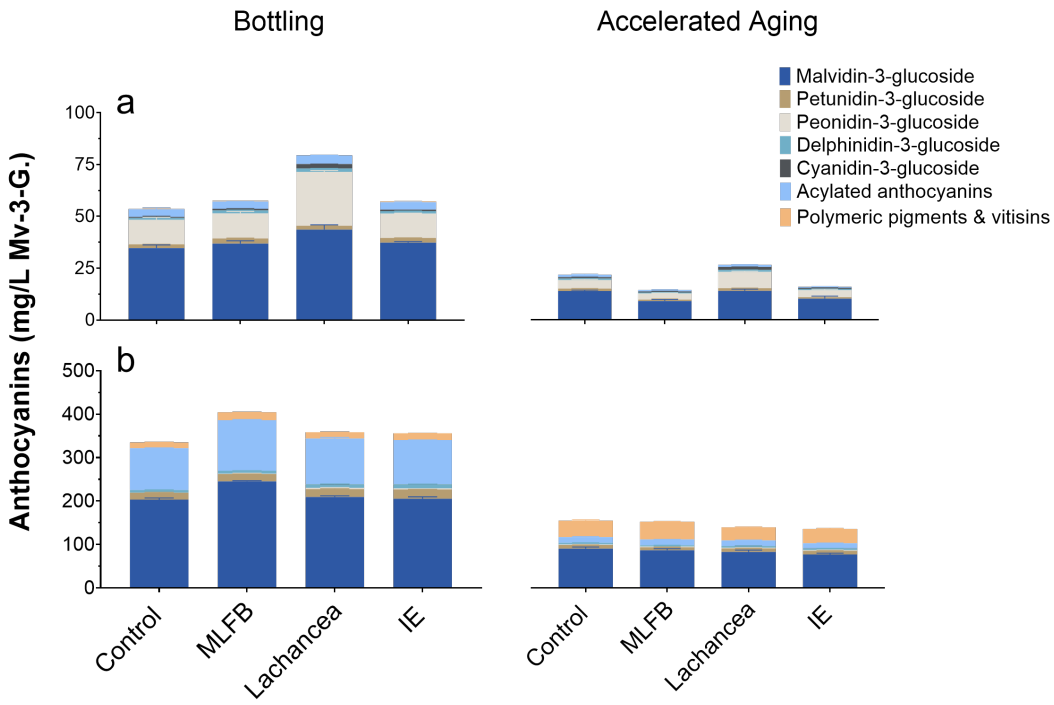

FIGURE 6. Evolution of anthocyanins and anthocyanin-derived pigments at bottling and following accelerated ageing of a) Nebbiolo and b) Tannat wines. Data points represent average values between three replicates (n = 3) of each treatment and bars represent standard error of the mean.

5. Monomeric flavan-3-ols

The evolution of monomeric flavan-3-ols in Nebbiolo and Tannat wines is shown in Figure 8. In Nebbiolo wines after 3 months of bottle ageing and accelerated ageing, there were significant treatment effects on the composition of monomeric flavan-3-ols (Table S7). Control wines showed higher concentrations of dimers compared to MLFB and Lachancea wines (36 % and 28 % higher, respectively). Trimers were highest in control and IE wines (2.8 *vs* 2.0 mg/L in MLFB and Lachancea wines), while sulfonated tannins were highest in control wines compared to all other treatments (0.8 *vs* 0.5 mg/L). Overall, total flavan-3-ol concentrations were 24 % and 19 % higher in control wines compared to MLFB and Lachancea wines, respectively. After accelerated ageing, control wines had 34 % and 32 % higher epicatechin concentrations compared to MLFB and IE wines, respectively. IE wines had the highest concentrations of dimers compared to all other treatments (21 % higher than the control), while Lachancea wines had 18 % lower trimer concentrations compared to the control. Total flavan-3-ol concentrations after accelerated ageing were highest in control and IE wines compared to MLFB wines (29 % and 22 % higher, respectively). These results suggest that MLFB and Lachancea wines had reduced monomeric flavan-3-ol concentrations compared to control wines, and that IE did not have an impact on flavan-3-ol concentrations compared to the control, and was the acid management strategy that was most effective at preserving flavan-3-ols in Nebbiolo wines. Previous studies have also found wines inoculated with *Lachancea thermotolerans* to have lower concentrations of flavan-3-ols compared to wines inoculated with *Saccharomyces cerevisiae* (Lei *et al*., 2025; Mucalo *et al*., 2023).

In Tannat wines after 3 months of bottle ageing, treatment effects on monomeric flavan-3-ols differed from those observed in Nebbiolo (Table S7). Epicatechin concentrations were higher in control and IE wines (4.5 and 3.9 mg/L, respectively) compared to MLFB and Lachancea treatments (2.9 and 3.0 mg/L, respectively). Dimer concentrations were highest in control and Lachancea wines (13.2 and 13.0 mg/L, respectively) compared to MLFB and IE wines (11.7 and 12.0 mg/L, respectively). However, total flavan-3-ol concentrations did not differ significantly between treatments at this timepoint. Following accelerated ageing, trimers were 29 % higher in control and IE wines compared to Lachancea wines, and sulfonated tannins remained highest in MLFB wines compared to all other treatments (90 % higher than the control). A statistically significant difference was found in total flavan-3-ol concentrations, with control wines having 21 % higher concentrations compared to Lachancea wines.

A two-way ANOVA summarised significant treatment effects at 3 months of bottle ageing (Table S8). There were significant treatment effects for several compounds including epicatechin, dimers, and trimers. Significant varietal × treatment interactions (Table S11) were observed for epicatechin gallate, dimers, trimers, and sulfonated tannins, indicating that treatment effects differed between Nebbiolo and Tannat for these compounds. Following accelerated ageing, treatment effects remained for several compounds, including epigallocatechin, epicatechin, dimers, and trimers (Table S8). Additionally, no significant varietal × treatment interactions were found after accelerated ageing (Table S8), indicating more consistent treatment effects across varietals over time.

6. Volatile composition of the wines

The acid management treatment resulted in significant differences in the concentration of key volatile compounds, as shown by the OAVs of these aroma compounds in both Nebbiolo and Tannat wines, assessed both at 3 months of bottle ageing and after accelerated ageing (Table S9). Across both varietals, Lachancea wines exhibited lower overall volatile concentrations, while MLFB and IE wines appeared to retain higher levels of volatiles following accelerated ageing. At 3 months of bottle ageing, Lachancea wines consistently showed lower OAVs for multiple esters across both varietals, including ethyl butyrate, ethyl isovalerate, ethyl hexanoate, and ethyl *n*-octanoate (Figure 9). Lachancea wines also had lower OAVs for isoamyl acetate and β-ionone in Nebbiolo wines (50 % and 26 % lower than the control, respectively). In Nebbiolo wines, control and MLFB treatments generally had the highest OAVs for ethyl butyrate and ethyl isovalerate, followed by IE wines, while Lachancea wines had the lowest values for all esters with OAVs greater than 1 (Figure 9). In Tannat wines, IE wines had the highest ethyl isovalerate OAVs (57 % higher than the control) at 3 months of bottle ageing, while Lachancea wines again had the lowest OAVs After accelerated ageing, Lachancea wines consistently retained the lowest OAVs for ethyl butyrate, ethyl isovalerate, isoamyl acetate, ethyl hexanoate, and ethyl *n*-octanoate across both varietals (Table S9).

These results suggest that while *Lachancea thermotolerans* indeed acts as a beneficial acidifying agent, the Lachancea treatment in this study was also associated with lower ester OAVs. Previous studies have also reported lower concentrations of isoamyl acetate, ethyl hexanoate, hexyl acetate, ethyl octanoate, and ethyl decanoate in Lachancea wines compared to those inoculated with *Saccharomyces cerevisiae* (Sánchez-Suárez *et al*., 2025; Sánchez-Suárez *et al*., 2026). A study on Merlot wines also found that wines inoculated *with Lachancea thermotolerans* had lower concentrations of ethyl butanoate, ethyl hexanoate, and ethyl octanoate compared to those inoculated with *Saccharomyces cerevisiae* (Hranilovic *et al*., 2021). A previous study investigating the effects of malolactic fermentation on volatile composition found that wines where MLF was blocked retained higher concentrations of isoamyl acetate, hexyl acetate, ethyl octanoate, and ethyl decanoate compared to those that did go through MLF (Prusova *et al*., 2024). This is consistent with the results we found showing MLFB wines have higher ester OAVs and preserve esters more compared to the other treatments.

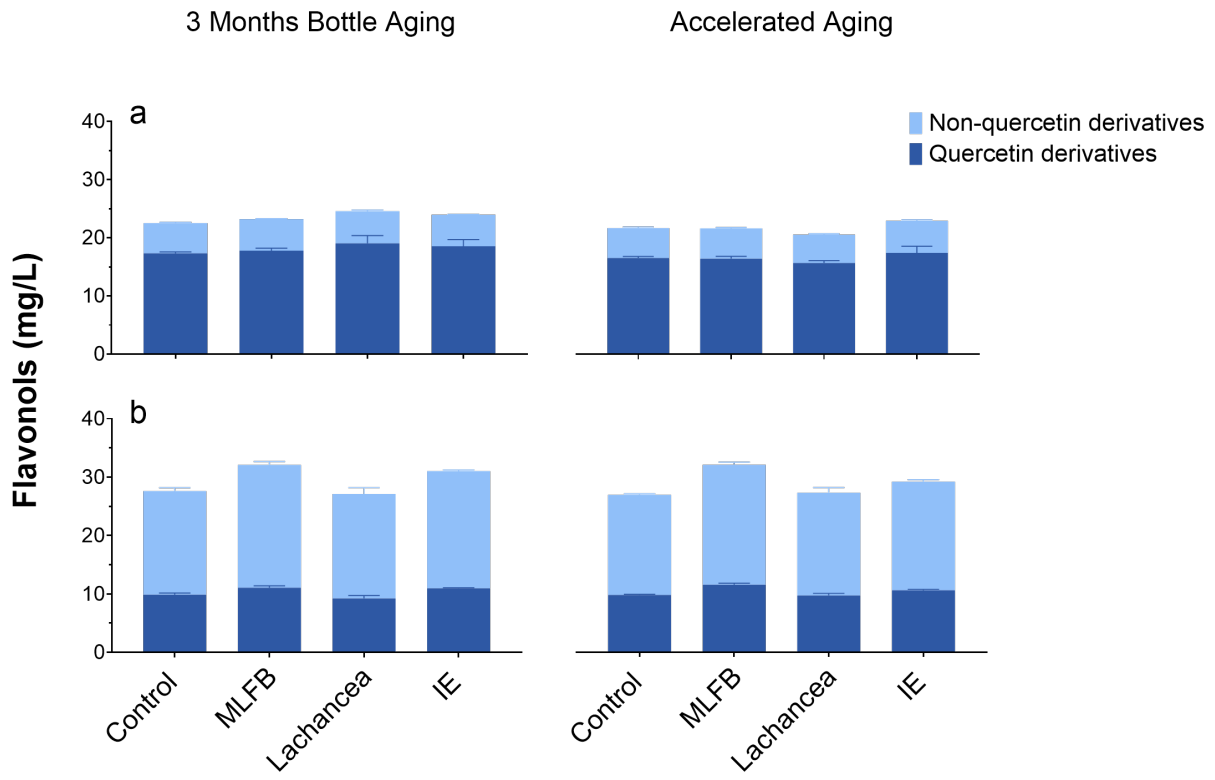

FIGURE 7. Evolution of flavonols at 3 months of bottle ageing and following accelerated ageing of a) Nebbiolo and b) Tannat wines. Data points represent average values between three replicates (n = 3) of each treatment and bars represent standard error of the mean.

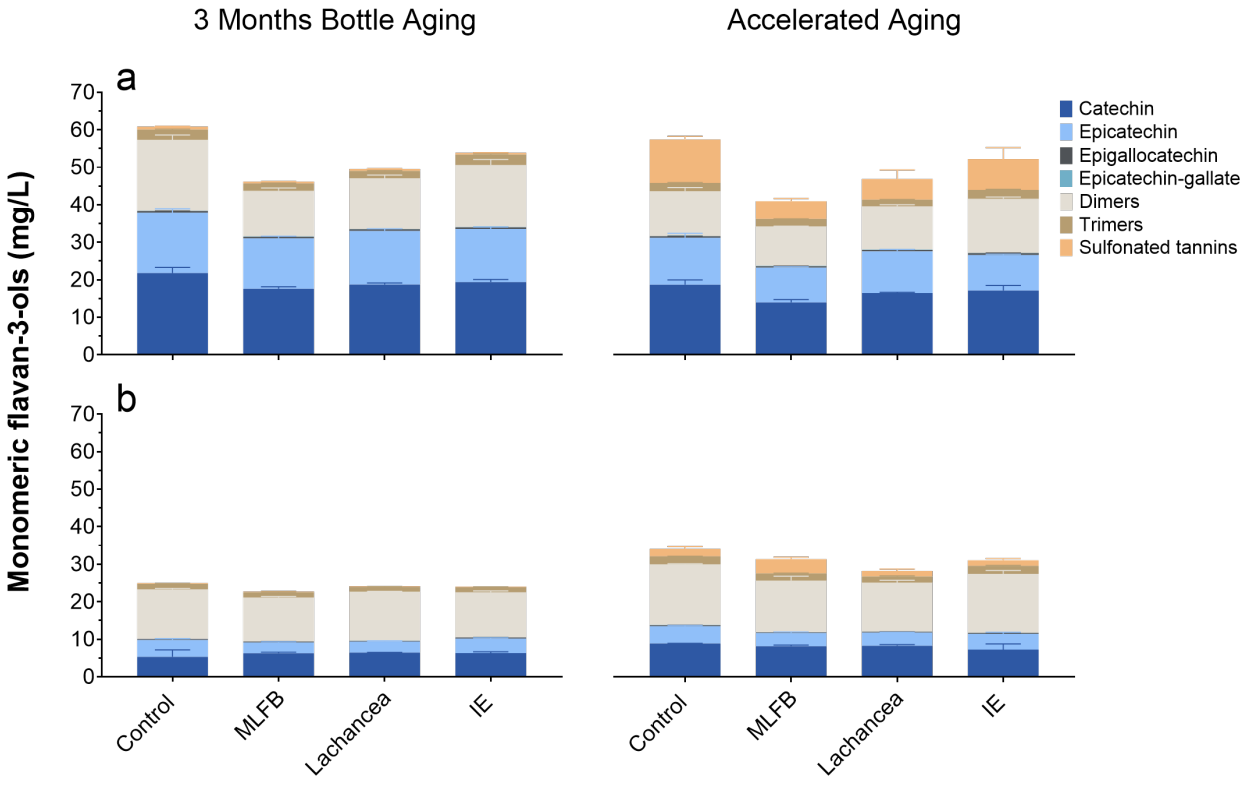

FIGURE 8. Evolution of monomeric flavan-3-ols in a) Nebbiolo and b) Tannat wines at 3 months of bottle ageing and following accelerated ageing. Data points represent average values between three replicates (n = 3) of each treatment, and bars represent the standard error of the mean.

7. Sensory analysis

Pivot© Profile analysis, using control wines as pivot, revealed clear sensory differences between acid management treatments in Nebbiolo wines (Figure 10). MLFB and IE wines showed more colour saturation compared to Lachancea wines. This is consistent with the higher total colour and polymeric pigment concentrations measured at bottling and after accelerated ageing in MLFB and IE wines (Figures 3 and 5). Furthermore, Lachancea wines were perceived as having the lowest astringency, whereas Control wines were comparatively more bitter (Figure 10). This could be attributed to differences in phenolics of Nebbiolo wines at 3 months of bottle ageing, which was the time point closest to when sensory analysis was conducted. Control wines had 51 % higher tannins than Lachancea wines (Figure 4), which contribute to astringency (Kallithraka *et al.*, 2011; Ma *et al.*, 2014). Control wines also had 24 % and 22 % fewer polymeric pigments than MLFB and IE wines, respectively (Figure 4). Previous studies suggest that polymeric pigments may contribute to the perception of a softer astringency (Casassa *et al.*, 2022), which could explain differences in astringency perception between control, MLFB, and IE wines. Lachancea wines were associated with more acidity and more purple hue. These findings are consistent with Lachancea wines' higher TA, which is directly related to perception of acidity.

Time-intensity descriptive analysis on Nebbiolo wines showed that Lachancea wines were perceived as having more red fruit flavour on the palate compared to control and MLFB wines (Figure 11). Lachancea wines had a significantly higher area under the curve (AUC) compared to control and MLFB treatments (161 intensity units *vs* 124 and 122, respectively), but not relative to IE wines (Table S10). Lachancea wines also had the highest peak value compared to control and MLFB treatments (7.4 *vs* 6.2 and 6.3, respectively), but not relative to IE wines (Table S10). This is contradictory to Lachancea wines having lower OAVs compared to other treatments for several ester compounds in Nebbiolo wines (Figure 9), suggesting that increased acidity may enhance red fruit perception. Previous sensory analysis studies have also found that inoculation with *Lachancea thermotolerans* can increase the perception of certain fruit aromas compared to *Saccharomyces cerevisiae* (Hranilovic *et al.*, 2022). Previous studies have also found that wines inoculated with *Lachancea thermotolerans* are perceived as "fresher" by panellists compared to wines inoculated with *Saccharomyces cerevisiae*, which could also be associated with and enhance the perception of "red fruit" on the palate (Morata *et al.*, 2018). Lachancea wines also had less tannin (Figure 4) and less perceived astringency (Figure 10). Being Nebbiolo a naturally highly tannic varietal, the comparatively higher astringency in other treatments could have diverted panellists' attention from the fruit flavour on the palate compared to Lachancea wines, resulting in higher fruit intensity ratings for the latter.

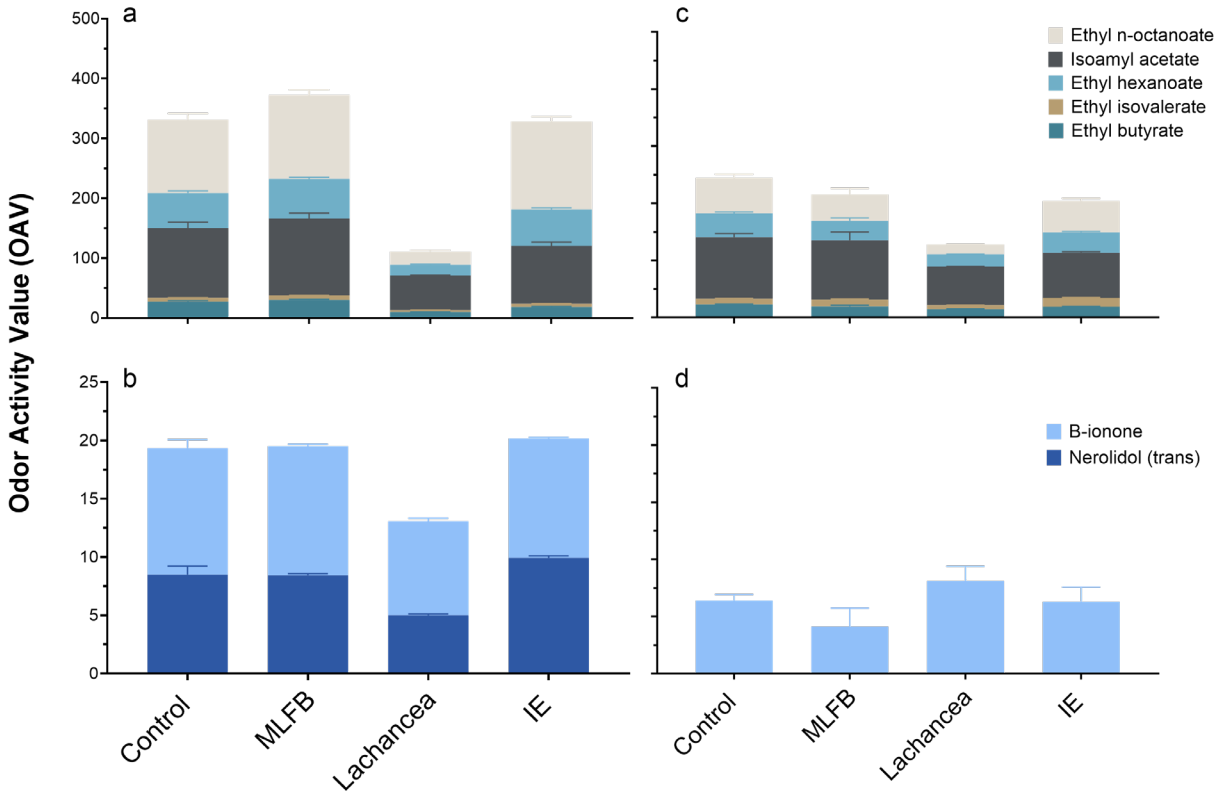

FIGURE 9. Ester and terpene odour activity values for a, b) Nebbiolo and c, d) Tannat wines measured at 3 months of bottle ageing. Error bars indicate standard error of the mean. Data points represent average values between three replicates (n = 3) of each treatment, and bars represent standard error of the mean.

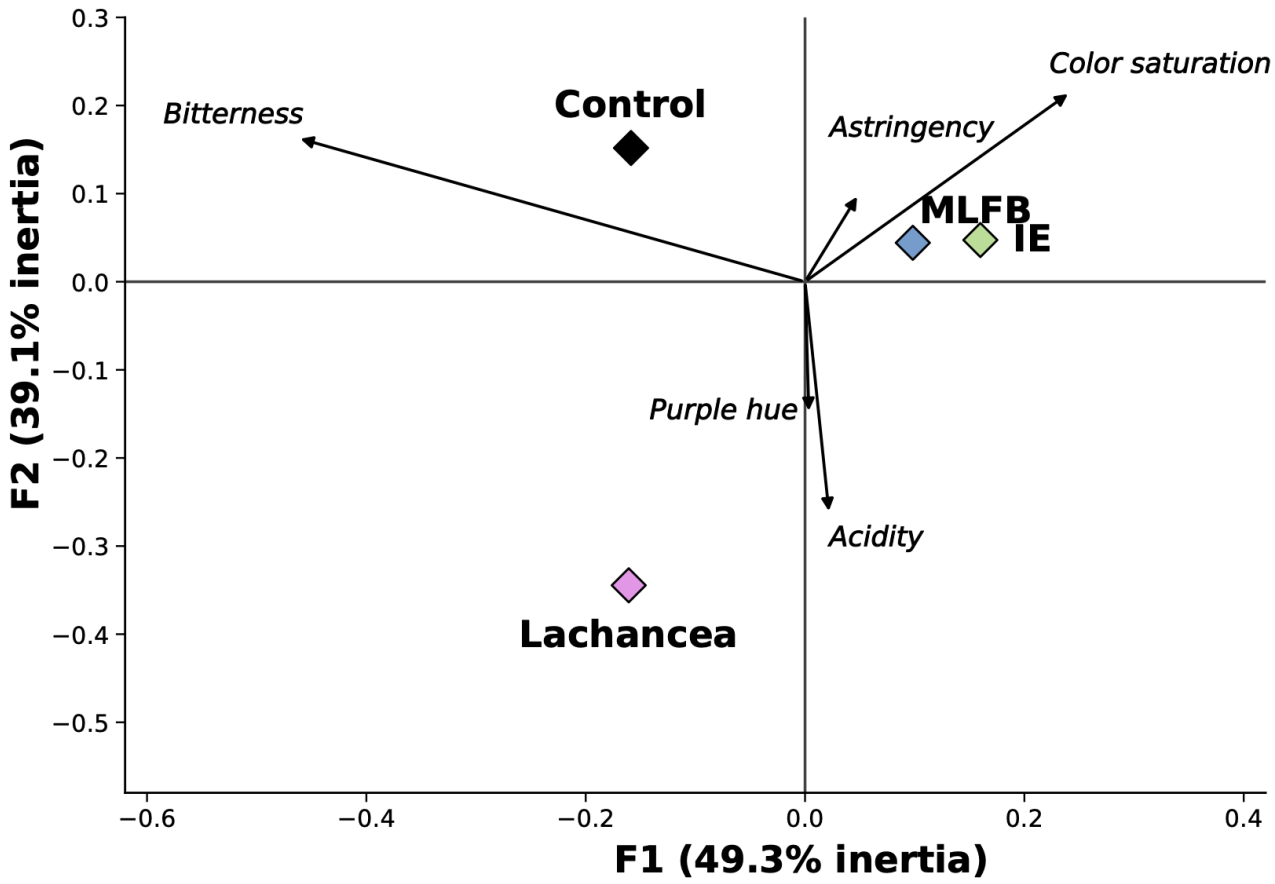

FIGURE 10. Correspondence analysis plot of Nebbiolo wines using data generated from Pivot© Profile (*n* = 10). The diamond-shaped forms represent the centroids of each distribution.

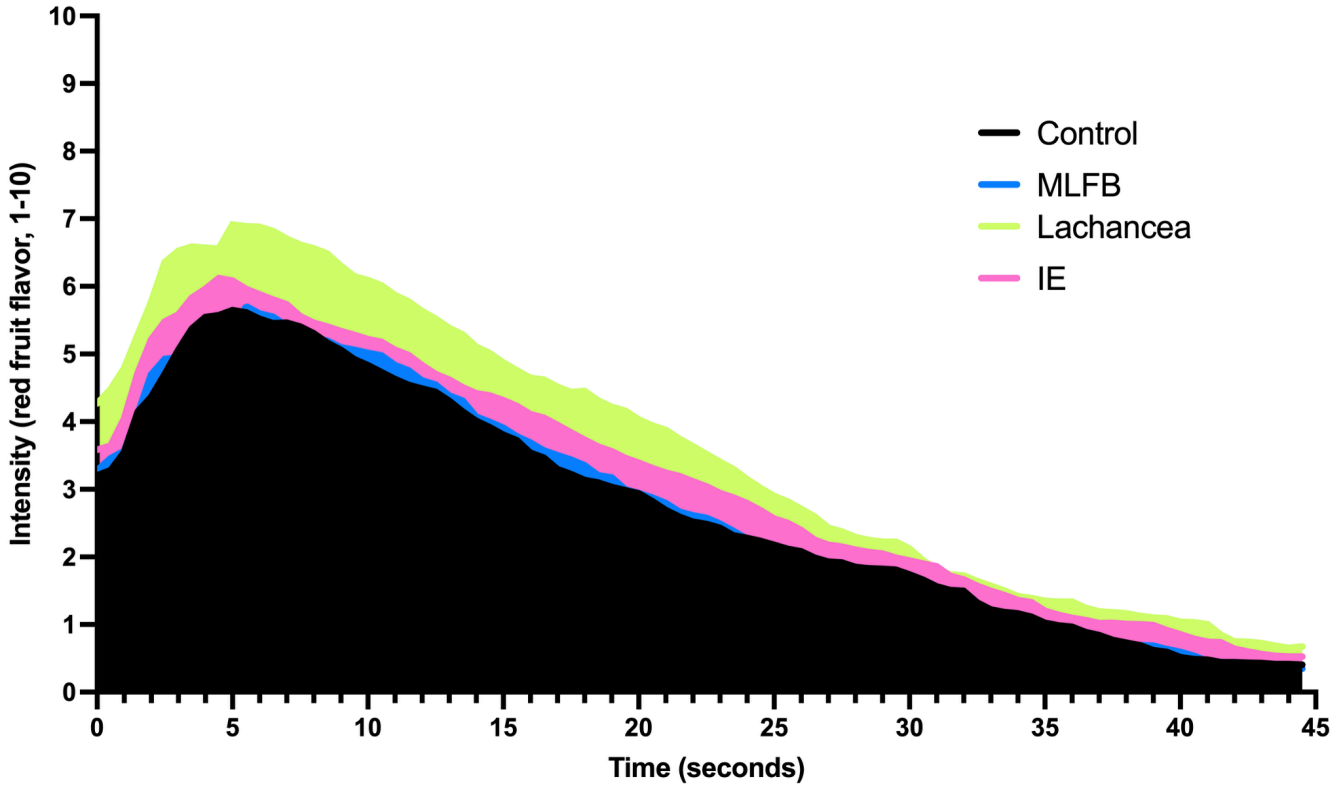

FIGURE 11. Time-intensity curves of perceived red fruit flavour on the palate post-expectoration in Nebbiolo wines across 45 seconds for each treatment. Curves represent the panel means (*n* = 11).

CONCLUSIONS

The present study has shown that various acid management strategies, including blocking MLF, inoculation with *Lachancea thermotolerans*, and ion exchange treatment with back-blending, produced differing chemical, phenolic, and sensory outcomes across Nebbiolo and Tannat wines from the Central Coast of California. Across both varietals, ion exchange was the most effective treatment at lowering pH and cation concentrations, especially K^+, making it a useful tool when potassium concentrations are major contributors to elevated pH in wine. Blending with IE wine also resulted in averaging effects for titratable acidity and ethanol. Blocking MLF emerged as a useful strategy in wines where the priority is preserving malic acid, maintaining a fresher profile, and improving colour retention and ageing potential. Overall, blocking MLF and ion exchange appear as viable strategies that can be used to help preserve colour throughout bottle ageing, as they contribute to a lower pH while retaining tannins. This was further supported by sensory analysis of the Nebbiolo wines, where MLFB and IE wines showed higher perceived colour saturation.

Wines made with *Lachancea thermotolerans* showed lower oxidation-reduction potential during alcoholic fermentation relative to fermentations with *Saccharomyces cerevisiae*, potentially due to the production of certain nucleophiles such as glutathione, which is a key effector of ORP. The mechanisms responsible for these differences require further investigation. These wines also showed the highest titratable acidity and, accordingly, the highest perceived acidity in sensory analysis of Nebbiolo wines, highlighting the effectiveness of using this yeast as a tool for biological acidification. Lachancea wines also consistently exhibited lower tannin (and, concurrently, lower astringency in Nebbiolo), polymeric pigment, and total phenolic concentrations, which suggests a tradeoff between increased acidity and lower phenolics when using *Lachancea thermotolerans*. Lachancea wines also consistently had the lowest OAVs for esters across both Nebbiolo and Tannat. However, time-intensity sensory analysis on Nebbiolo wines showed that panellists perceived Lachancea wines to have the most "red fruit" flavour on the palate. We attribute this to the lower astringency in wines fermented with *Lachancea thermotolerans* compared to the other treatments, creating less competition on the palate to perceive the fruit, as well as the higher perceived acidity and freshness of Lachancea wines. These results suggest that inoculation with *Lachancea thermotolerans* can be a useful tool when the priority is increasing freshness, especially in warm-climate, low-TA fruit, and the stylistic goal is geared towards early-to-market wines.

The bulk of these findings demonstrate that each acid management strategy produced distinct chemical and sensory outcomes that may be selected depending on the grape variety, initial fruit composition, and stylistic goals of the winemaker. By comparing three acid management strategies across two distinct red grape varieties throughout winemaking and ageing, this study provides practical guidance for selecting an acid management approach based on specific winemaking goals. It also helps to expand our understanding of their effects on wine chemistry, phenolic composition, volatile composition, and sensory properties.

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