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Dual-temperature dual-state fermentation: A novel approach to improve aroma and color characteristics of Marselan wines

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A R T I C L E I N F O

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A B S T R A C T

This study examined the effects of the Dual-Temperature Dual-State Fermentation (DTSF) technique on the chemical and sensory characteristics of industrial-scale 'Marselan' wine. Compared to the control wine, DTSF wine exhibited greater color intensity (chroma) attributed to higher levels of anthocyanin derivatives and copigments, along with a lower pH. Furthermore, DTSF wine retained higher concentrations of grape-derived aroma volatiles, including C6/C9 compounds, terpenoids, and norisoprenoids, and elevated levels of fermentation-derived esters (notably ethyl esters), contributing to a more intense fresh fruit aroma. Additionally, the DTSF technique had a minimal impact on condensed tannins and mouthfeel. This study confirms the viability of the DTSF technique for producing high-quality red wines and highlights its potential for the production of wines with diverse sensory profiles.

1. Introduction

Fermentation temperature is a critical factor in winemaking, influencing not only the rate and efficiency of fermentation but also the sensory characteristics of the wine, such as aroma, taste, and color. Specifically, temperature regulates two key processes: maceration efficiency, which governs phenolic extraction, and yeast metabolism, which drives aroma development. To achieve distinct wine styles, winemakers often adjust fermentation temperatures. For traditional red wines, higher temperatures (25–30 °C) are used to enhance phenolic extraction from grape skins, resulting in wines with richer color and structure (Casassa et al., 2023; Casassa & Harbertson, 2014). While higher temperatures may theoretically enhance the extraction of volatile compounds and their precursors, they also increase evaporation, leading to significant losses of these compounds (Du et al., 2022; Rollero et al., 2015). Moreover, the shorter fermentation durations associated with high temperatures can produce coarse and underdeveloped aromas, ultimately compromising the wine's aromatic quality (Ntuli et al., 2022).

While low-temperature fermentation is unsuitable for skin maceration, this limitation is irrelevant in white wine production, where the extraction of anthocyanins and tannins is unnecessary. In white wine vinification, after destemming and crushing, the must is pressed, and the clear juice is fermented at low temperatures (15–20 °C). This approach preserves a higher concentration of grape-derived aromatic compounds and extends fermentation duration, contributing to the retention of delicate and complex aroma profiles (Kanellaki et al., 2014; Massera et al., 2021). Additionally, there is substantial evidence indicating the significant influence of low temperatures on primary and secondary yeast metabolism, affecting compounds including pyruvate, ethanol, glycerol, acetic acid, and notably, various aromatic compounds (Beltran et al., 2008; Tai et al., 2007; J. Llauradó et al., 2002, 2005; Tilloy et al., 2014). Although the effects of low-temperature fermentation on fatty acids, higher alcohols, and acetates vary across studies (Beltran et al., 2008; Deed et al., 2017; Massera et al., 2021; Torija et al., 2003; Tronchoni et al., 2012), a consistent finding is the enhanced synthesis of ethyl esters under low temperatures. This increase in ethyl esters enhances fruit flavors and positively influences the wine's aromatic profile (Du et al., 2022, 2024; Gamero et al., 2013; Massera et al., 2021).

The use of clarified juice in white wine fermentation promotes the

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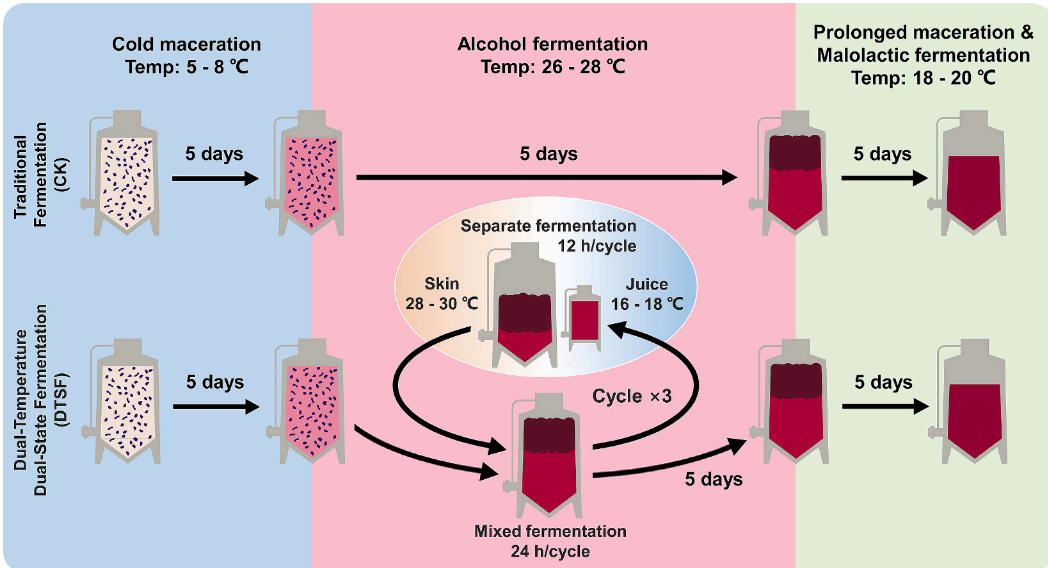

Fig. 1. Comparison of the Dual-Temperature Dual-State Fermentation (DTSF) process and the traditional red wine vinification process (CK). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

synthesis of ester compounds. In winemaking practices, fermenting juice with low turbidity favors the development of fruity aromas (Vernhet et al., 2016). Research also shows that an increase in grape insoluble material decreases the ester content in Savatiano and Batiki wines (Karagiannis & Lanaridis, 2002). This effect may be attributed to an esterase present in grape tissues, which limits the accumulation of esters produced by yeast (Casalta et al., 2016). Modern red winemaking techniques, such as flash détente and pre-fermentation heat treatment, involve sufficient extraction of phenolic compounds from grape skins during maceration, enabling fermentation with clarified juice instead of whole grape solids (Tong et al., 2024). In red wines produced using these techniques, fermenting clarified juice results in higher levels of esters and fatty acids than in wines fermented with grape solids (Geffroy et al., 2015; Ntuli et al., 2022).

While the individual effects of high-temperature (phenolic extraction) and low-temperature (aroma retention) fermentation are well documented, no existing methodology systematically integrates these opposing temperature requirements to optimize both phenolic and aromatic profiles in red winemaking. Modern techniques like flash détente enable clarified juice fermentation but lack dynamic temperature-state regulation to resolve the trade-off between phenolic extraction and volatile compound synthesis and preservation. To bridge this gap, we developed a novel fermentation technique called the Dual-Temperature Dual-State Fermentation (DTSF). This method consists of two fermentation phases with distinct temperatures and states: low-temperature fermentation of clarified juice and high-temperature fermentation with grape skins. During AF (Alcoholic fermentation), a portion of the must is separated and fermented without skins at a lower temperature to enhance aroma, while the remaining must undergoes skin-contact fermentation at a higher temperature to maximize color and phenolic extraction. These portions are then recombined, and this cycle is repeated multiple times during AF. This method aims to produce wine that combines the enhanced aroma complexity of low-temperature, skinless fermentation with the intensified color and phenolic structure resulting from high-temperature, skin-contact fermentation.

This study investigates the volatile and phenolic compounds of ‘Marselan’ red wine produced using the DTSF process on an industrial scale. Additionally, the sensory characteristics of DTSF wines are clearly defined to determine the potential of this technique in producing high quality red wines.

2. Materials and methods

2.1. Standards and reagents

Beijing Chemical Works (Beijing, China) provided analytical-grade chemicals, such as acetone, resorcinol, sodium acetate, anhydrous copper sulfate, disodium hydrogen phosphate, potassium hydrogen phthalate, sodium hydroxide, citric acid, glucose, and sodium chloride. Honeywell (Morris Plains, NJ, USA) provided chromatographic-grade solvents (methanol, ethanol, and acetonitrile; purity ≥99.9 % for all), while Reagent World (Newark, NJ, USA) provided formic acid (≥99 %). Supplementary Table 1 contains the entire set of standards for phenolic and aroma chemicals.

2.2. Dual-temperature dual-state fermentation technique (DTSF)

In 2023, ‘Marselan’ (*Vitis vinifera* L.) grape clusters were harvested from commercial vineyards in the Penglai region (120°52′E, 37°44′N). The grapes were manually harvested at full ripeness (24.6°Brix), destemmed, and sorted to remove diseased fruit and immature berries. After crushing, the must was transferred to 5000 L fermenters. Total SO_2 was adjusted to 45 mg/L using a potassium metabisulfite solution (6 % *w/v*), and 20 mg/L pectinase (Vinozym® Vintage FCE, Novozymes) was added. The must underwent cold maceration at 5–8 °C for 5 days, with four closed pump-overs per day, each circulating approximately 25 % of the total volume.

For the DTSF process, on the fifth day of cold maceration, the must was rewarmed to 26 °C, and alcoholic fermentation (AF) was initiated by adding 200 mg/L of active yeast (LALVIN ICV D254, Lallemand Oenology, Bordeaux, France). When the density decreased by 0.03 (equivalent to a 7° Brix reduction), 800 L of juice was transferred from the red wine fermenter to a white wine fermenter for separate fermentation. The juice in the white wine fermenter was fermented at a lower temperature (16–18 °C) for 12 h, while the remaining must in the red wine fermenter continued fermentation at a higher temperature (28–30 °C). The 12-h duration was chosen based on preliminary experiments, which indicated that this period allowed sufficient extraction of aromatic compounds while maintaining fermentation kinetics. After 12 h, the fermenting juice from the white wine fermenter was transferred back to the red wine fermenter, where it was mixed with the must and fermented for an additional 24 h at 26–28 °C. This entire process—transfer, separate fermentation, and re-mixing—was repeated three times, constituting the Dual-Temperature Dual-State Fermentation (DTSF) technique.

During AF, the must was pumped over six times daily, circulating approximately 25 % of the total volume each time. AF was completed within 5 days, followed by prolonged maceration. At this stage, 6 mg/L of lactic acid bacteria (EnartisML Uno, Enartis S.p.A, Lecco, Italy) was inoculated to initiate malolactic fermentation (MLF), which was conducted at a controlled temperature range of 18–20 °C for 3 days. After MLF, the wine was racked, and the free-run and pressed wines were blended and allowed to continue MLF for an additional 2 days.

The control wine was produced under the same conditions as the DTSF wine, except that the must was not subjected to the separate fermentation process during AF. A detailed schematic of the DTSF process is provided in Fig. 1. At the end of AF and MLF, wine samples were collected after pumping over to ensure homogeneity within the fermenter. For each replicate, three 500 mL samples were collected and stored at −40 °C for subsequent chemical analysis. Additionally, six 750 mL bottles wine were stored in a cellar (16–18 °C and 70 % humidity) for one month prior to sensory evaluation.

2.3. Physicochemical parameter measurement

The wine samples were first subjected to centrifugation at 4000 ×*g* for 8 min. Basic physicochemical parameters including pH, volatile acidity, titratable acidity, residual sugar and ethanol content, were determined by a rapid-scanning infrared Fourier transform spectrometer (Foss WineScan, Foss Electric, Hillerød, Denmark). Each sample was analyzed in triplicate.

2.4. Color measurement

The color characteristics of the wines were evaluated using the CIELab method (Ayala et al., 1997). Initially, wine samples were filtered through a 0.45 μm polyethersulfone filter (Jinteng Experimental Equipment Co., Ltd., Tianjin, China) and transferred to glass cuvettes with a path length of 2 mm. A UV–Vis spectrophotometer (UV-2450; Shimadzu Co., Ltd., Kyoto, Japan) was used for detection. The parameters L^*, a^*, and b^* were recorded, while C^*ab and h values were computed. Each sample was analyzed in triplicate.

2.5. Quantitative analysis of organic acids

Organic acids in wine were qualitatively and quantitatively analyzed by an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA) (Zhang, Tang, et al., 2022). Separation utilized a 5 mM H_2SO_4 solution as the mobile phase, with a flow rate of 0.6 mL/min on a 300 mm × 7.8 mm Aminex HPX-87H ion exchange column (Bio-Rad Laboratories, Hercules, CA). Detection occurred at 214 nm using a UV detector, with the column temperature maintained at 60 °C. Samples were filtered through a 0.22 μm membrane before analysis, and all were analyzed in triplicate.

2.6. Quantitative analysis of phenolic compounds

Phenolic compounds in the wine were analyzed using an Agilent 1200-6410B High-Performance Liquid Chromatography/Triple Quadrupole Tandem Mass Spectrometer (HPLC-QqQ-MS/MS, Agilent Technologies, Santa Clara, CA). The separation of phenolics was carried out using an Agilent Poroshell 120 EC-C18 column (150 mm × 2.1 mm, 2.7 μm). The mobile phase consisted of 0.1 % formic acid in water (A) and a 50:50 (*v*/v) mixture of acetonitrile and methanol containing 0.1 % formic acid (B).

2.6.1. Anthocyanins and non-anthocyanin phenolic compounds

We followed our previously reported method (Li et al., 2018) for the analysis of anthocyanins and non-anthocyanin phenolics. Wine samples were filtered using a 0.22 μm polyethersulfone membrane prior to analysis. The column was set to a temperature of 55 °C with a flow rate of 0.4 mL/min, and the injection volume was 1 μL. A linear gradient was applied for solvent B, starting from 10 % and increasing to 46 % over a span of 28 min, then returning to 10 % within 1 min, followed by a column re-equilibration at 10 % for 5 min.

The analysis of malvidin derivatives followed our previously reported method (Zhang et al., 2020). A linear gradient elution program was employed using solvent B with the following proportions (*v*/v): 0 % for 1 min, then increasing to 25 % for 3 min, from 25 % to 30 % for 15 min, and from 30 % to 100 % for 20 min. The flow rate was set to 0.3 mL/min with an injection volume of 10 μL. Mass spectrometry was performed in the positive ion mode, while maintaining identical conditions to those previously described for the other parameters.

The ion source and MRM parameters were consistent with those reported in our previous publication (Li et al., 2018; Zhang et al., 2020). The qualitative and quantitative analysis methods, as well as the establishment of external calibration curves, were also performed following the procedures outlined in our previous work (Yao et al., 2024).

2.6.2. Tannins

Solid-phase extraction (SPE) combined with an Agilent UHPLC-FLD-Q-TOF was used to analyse the concentration and degree of polymerisation (DP) of tannins in wine. To extract condensed tannin, 5 mL sample was applied to a pre-conditioned Oasis HLB solid-phase extraction cartridge (3 cc/60 mg, Waters, MA, USA) for purification, then eluted using 20 mL of a solution composed of 80 % acetone, 19.5 % water, and 0.5 % acetic acid (*v*/v/v). The dried residue was dissolved in 1 mL of a 14 % ethanol/water mixture (*v*/v) for analysis. The separation of tannins was carried out on a Develosil Diol 100 Å (250 × 4.6 mm, 5 μm) column. Detailed conditions can be found in prior literature (Robbins et al., 2013).

2.7. Quantitative analysis of aroma compounds

Aroma compounds were analyzed using headspace solid-phase microextraction (HS-SPME) combined with gas chromatography–mass spectrometry (GC–MS), as outlined in our previous studies (Lan et al., 2019; Tong et al., 2024).

For sample preparation, 5 mL of wine was mixed with 10 μL of 4-methyl-2-pentanol (internal standard, 1.0086 g/L) and 1.0 g NaCl, followed by preheating at 40 °C for 30 min in a sealed vial. A DVB/CAR/PDMS SPME fiber (50/30 μm; Supelco, Bellefonte, PA, USA) was then exposed to the headspace under agitation (500 rpm) for 30 min at 40 °C, followed by desorption for 8 min in the GC injector. Volatile compounds were separated on an HP-INNOWAX capillary column (60 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA, USA) using an Agilent 6890 GC with helium carrier gas at 1 mL/min, and analyzed by an Agilent 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The MS operated in electron ionization (EI) mode at 70 eV, with ion source and quadrupole temperatures set to 250 °C and 150 °C, respectively, and a full-scan mass range of m/z 30–350. Additional parameters for compound identification/quantification were consistent with our prior methodology (Lan et al., 2019; Tong et al., 2024).

2.8. Sensory evaluation

Ethical approval for the participation of human subjects in this study was granted by the Research Ethics Committee of China Agricultural University (approval number: CAUHR-20231103). The sensory panel comprised 15 individuals (6 men and 9 women, aged 23 to 30), all of whom were students or staff from the Center for Viticulture and Enology (CFVE). Participation in sensory evaluation was voluntary, with informed consent obtained for both the sensory data collection and the

Table 1
Physicochemical parameters of the wines made by Dual-Temperature Dual-State Fermentation (DTSF) and traditional fermentation (CK) at the end of alcoholic fermentation (AF) and malolactic fermentation (MLF).

Physicochemical parameters	AF-CK	AF-DTSF	Significance[a]	MLF-CK	MLF-DTSF	Significance[a]
Ethanol (%,V/V)	14.61 ± 0.02	14.57 ± 0.03	NS	14.68 ± 0.02	14.62 ± 0.01	**
Residual sugar (g/L)	6.11 ± 0.05	7.59 ± 0.10	***	4.8 ± 0.04	6.73 ± 0.11	***
Titratable Acidity (g/L)	6.44 ± 0.02	6.63 ± 0.03	**	6.16 ± 0.02	6.23 ± 0.02	*
Volatile Acidity (g/L)	0.62 ± 0.01	0.58 ± 0	**	0.6 ± 0	0.64 ± 0	**
pH value	3.57 ± 0.01	3.43 ± 0	***	3.58 ± 0	3.45 ± 0.01	***

[a] *, **, ***, NS: Significant at $p \leq 0.05$, 0.01, 0.001, or not significant, respectively, student's *t*-test.

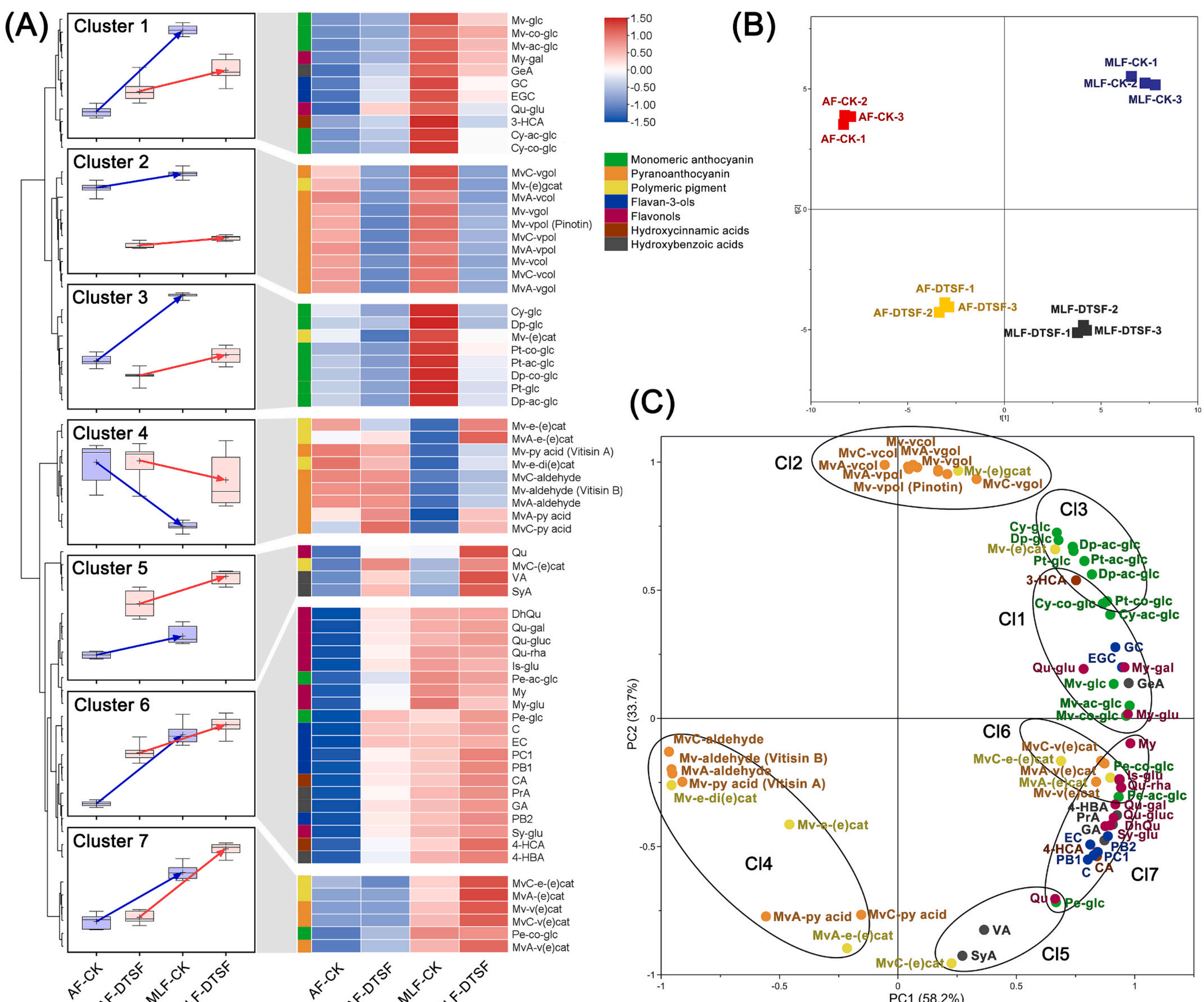

Fig. 2. Phenolic characteristics of CK and DTSF wines. (A) Cluster heatmap and boxplot of phenolic compounds at the end of alcoholic fermentation and malolactic fermentation, (B) PCA score plot, and (C) loading plot.

use of personal information. Prior to the formal evaluation, the panelists participated in four training sessions over one month. During training, they evaluated the wines' aroma and taste characteristics and identified five key aroma descriptors (green, fresh fruit, red fruit, black fruit, floral) and four taste descriptors (acidity, bitterness, astringency, and body). Each attribute was rated on a 10-point scale.

2.9. Statistical analysis

Statistical analyses were performed using Student's *t*-test (for pairwise comparisons) and one-way ANOVA (for multi-group comparisons) in R version 3.4.2. Heatmaps were created with TBtools (Chen et al., 2023). Additionally, PLS-DA was executed with SIMCA (version 14.1, Umetrics, Umeå, Sweden).

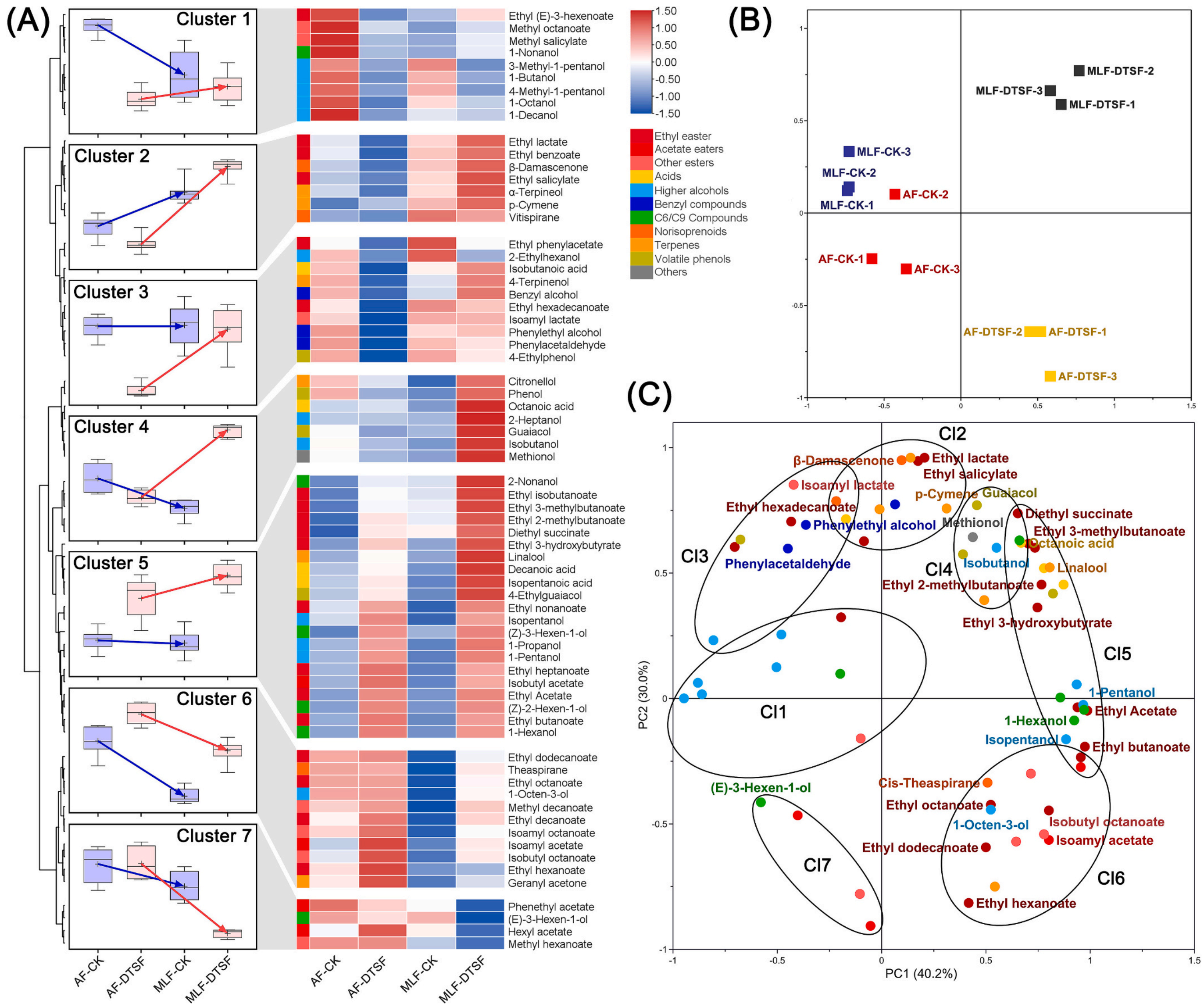

Fig. 3. Aroma characteristics of CK and DTSF wines. (A) Cluster heatmap and boxplot of volatile compounds at the end of alcoholic fermentation and malolactic fermentation, (B) PCA score plot, and (C) loading plot (Compounds with OAV values less than 0.01 were not labelled).

3. Results and discussion

3.1. Physicochemical parameters

The ethanol content, residual sugar (RS), titratable acidity (TA), volatile acidity (VA), and pH of the wines at the end of AF and MLF are presented in Table 1. DTSF wines exhibited similar ethanol concentrations but higher RS levels at both fermentation stages compared to the control wines. This difference may be attributed to the 36 h low-temperature fermentation (10 °C lower than the control) of 30 % of the fermenting liquid during the DTSF process. The lower temperature enhances yeast fermentation efficiency, enabling it to utilize less sugar to achieve an ethanol concentration comparable to that of the control (Tai et al., 2007).

Additionally, DTSF wine exhibited higher TA and lower pH values. Analysis of organic acid content (Table S2) revealed significantly lower tartaric acid levels in MLF-DTSF wines compared to the control, while succinic acid, lactic acid, and malic acid levels were notably higher. These results align with previous studies indicating that low-temperature fermentation increases malic and succinic acid concentrations Error! Bookmark not defined. Additionally, the higher succinic acid content in DTSF wines led to a 20-fold increase in diethyl succinate compared to the control (Table S4). Although volatile acidity (VA) differed significantly between DTSF and control wines, the trends were inconsistent across fermentation stages. However, VA levels in both wines remained below 0.9 g/L, a threshold that does not compromise wine quality (Vilela-Moura et al., 2011).

3.2. Phenolic compounds

Phenolic compounds are key determinants of wine color and flavour. A total of 41 anthocyanins were identified: 15 monomeric anthocyanins (including 5 non-acylated and 10 acylated anthocyanins and 26 derived anthocyanins (including 18 pyranoanthocyanins, and 8 polymeric pigments). Additionally, 7 flavan-3-ols, 11 flavonols, and 9 phenolic acids were also detected (Table S3). The profiles of these compounds across treatments and two fermentation stages are visualized in the clustering heatmap and PLS-DA, with compounds grouped into seven clusters (Fig. 2).

The compounds in Clusters 1, 2, and 3 are located in the first quadrant of the loading plot, indicating their highest concentrations in control wines at the end of MLF (Fig. 2C). Clusters 1 and 3 exhibit similar trends, with slight differences at the end of AF and a more pronounced increase in control wines after MLF compared to DTSF wines. These clusters include 12 monomeric anthocyanins. The observed differences during MLF, rather than AF, suggest that these compounds are influenced by prolonged maceration rather than initial maceration processes. The increase in monomeric anthocyanins during MLF aligns with the expected effects of prolonged maceration. In DTSF wines, anthocyanins are utilized for synthesizing higher levels of pyranoanthocyanins and polymeric pigments (Table S3). Cluster 2 compounds, including pinotins (Mv-vpol, Mv-vcol, and Mv-vgol) and their acylated forms, showed higher concentrations in control wines at the end of AF, a trend that persisted through MLF. It is hypothesized that in DTSF wines, anthocyanins preferentially participate in the formation of vitisin A and vitisin B, thereby reducing the availability of precursors for pinotin synthesis. Previous studies on 'Pinotage', 'Cabernet Sauvignon', and 'Marselan' have demonstrated that pinotin biosynthesis competes with other derived anthocyanins (Schwarz et al., 2003; Zhang et al., 2024; X.-K. Zhang et al., 2021). While pinotin accumulates primarily during aging (2–4.5 years), its synthesis during fermentation is limited. Therefore, despite higher levels of caffeic acid and 4-hydroxycinnamic acid in DTSF wines (Table S3), their contribution to pinotin biosynthesis may be more pronounced during the later stages of aging.

Cluster 4 includes acetaldehyde-mediated polymeric pigments, vitisin A, vitisin B, and their acylated forms. These compounds showed minor differences between groups at the end of AF. Their concentration decreases during MLF, however, the decrease is less pronounced in DTSF wines, resulting in higher concentrations. This trend aligns with the dependence of these compounds on pyruvate and acetaldehyde generated by yeast metabolism, as supported by previous research (Yao et al., 2024). In DTSF wines, the lower fermentation temperature in the white wine fermenter enhances the activity of yeast pyruvate kinase and pyruvate decarboxylase, increasing pyruvate and acetaldehyde production (Tai et al., 2007). Upon transfer back to the red wine fermenter, these molecules interact with anthocyanins, promoting the synthesis of vitisin A, vitisin B, and acetaldehyde-mediated polymeric pigments.

Clusters 5, 6, and 7 are located in the fourth quadrant of the loading plot, indicating higher concentrations in DTSF wines at the end of MLF. Cluster 5 includes quercetin, a coumaroylated polymeric pigment (MvC-(e)cat), and two hydroxybenzoic acids (VA and SyA). Cluster 6 comprises most flavonols, flavanols, and phenolic acids. The flavonols and flavanols were mainly extracted from grape pomace and showed higher levels in the DTSF wines at the end of AF. This increase is likely due to the higher temperature than the control was used to extract the skins for 36 h of the DTSF-AF process, which is consistent with previous studies showing that higher fermentation temperatures increase the extraction of these compounds (Casassa & Harbertson, 2014). Phenolic acids, primarily derived from grape pulp, can enter the wine without maceration. In grape berries, these phenolic acids mainly exist as tartaric esters, which are more readily hydrolyzed to release free phenolic acids under lower pH conditions (Lima et al., 2018; Virdis et al., 2021), possibly explaining their higher concentrations in DTSF wines.

Cluster 7 compounds, including three flavanyl-pyranoanthocyanins and two polymeric pigments, showed minimal differences at the end of AF but higher concentrations in DTSF wines after MLF. This may be related to the higher substrate levels (catechin and epicatechin) in DTSF wines (Table S3).

3.3. Volatile compounds

Volatile compounds play a crucial role in shaping the diverse aroma profile of wine. We identified 69 volatile compounds, including 30 esters, 4 organic acids, 12 higher alcohols, 5 terpenoids, 4 norisoprenoids, 6 C6/C9 compounds, 3 benzenoid compounds, 4 volatile phenols, and other aroma compounds (Table S4). The profiles of these compounds across treatments and fermentation stages were analyzed using cluster heatmaps and PLS-DA, resulting in seven distinct clusters (Fig. 3). Additionally, the odor activity values (OAV) of these compounds were calculated, with OAV > 1 indicating significant contributions to sensory perception (Dein et al., 2021).

Cluster 1 is located on the negative x-axis of the loading plot, indicating lower concentrations of these compounds in DTSF wines, particularly at the end of AF. This cluster includes six higher alcohols. It is hypothesized that the lower fermentation temperature in the white wine fermenter during the DTSF process suppresses the formation of higher alcohols, which is consistent with numerous studies (Beltran et al., 2008; Du et al., 2022; Gamero et al., 2013). However, the OAV values of these compounds are all below 1, suggesting minimal impact on wine aroma.

Clusters 2 and 3 show similar trends, with both positioned on the positive Y-axis. Compounds within these clusters generally increased significantly during MLF, except for those in Cluster 3 from the CK group, which remained stable. Cluster 2 includes three ethyl esters, two terpenoids, and two abundant norisoprenoids. Among these, only *p*-cymene and *β*-damascenone have OAV > 1, contributing floral and fruity aromas to DTSF wines. Cluster 3 contains compounds related to phenylalanine metabolism, including phenylacetaldehyde, benzyl alcohol, phenylethyl alcohol, ethyl phenylacetate, 4-ethylphenol. Their lower concentrations in DTSF wines at the end of AF may result from suppressed yeast metabolism of phenylalanine due to the lower fermentation temperature (Du et al., 2022; Gamero et al., 2013). However, these compounds subsequently increased in concentration during MLF in DTSF wines, reaching or even surpassing the levels in control wines. This suggests that phenylalanine not utilized by yeast during AF might be metabolized by lactic acid bacteria (Ardö, 2006).

Clusters 4 and 5 are positioned on the positive X-axis, indicating higher concentrations in MLF-DTSF wines. These clusters include three volatile phenols (including guaiacol, phenol, and 4-ethylguaiacol), which contribute smoky and horsey off-flavors to the wine. Their higher levels in DTSF wines are associated with higher phenolic acid content. Numerous studies have shown that the production of volatile phenols from phenolic acids is catalyzed by phenolic acid decarboxylase in lactic acid bacteria (Cappello et al., 2017; Virdis et al., 2021), explaining the increase of these compounds during MLF in DTSF wines. Cluster 5 consists of a large variety of compounds including fatty acids, higher alcohols, ethyl esters, C6/C9 compounds, and linalool. Most of these compounds were present in higher concentrations in DTSF wines at the end of AF and MLF. Our results align with many studies (Beltran et al., 2008; Du et al., 2022; Gamero et al., 2013; Ntuli et al., 2022) showing that low-temperature fermentation of clarified juice promotes ester accumulation. This finding is consistent with the objective of the DTSF process, which involves low-temperature fermentation of clarified juice in a white wine fermenter to enhance the fruity aroma in red wines. C6/C9 compounds and linalool primarily originate from the extraction during maceration and the release of glycosidically bound aromas. The higher levels of these compounds in DTSF wines can be attributed to two factors: i) lower pH value promotes hydrolysis of glycosidic state aromas (Liu et al., 2017), and ii) the lower temperature in the white wine fermenter during AF, which reduces the evaporation loss of these compounds (Rollero et al., 2015).

Cluster 6 and Cluster 7 are located on the negative Y-axis and include many ester compounds, such as isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate, which are significantly higher in DTSF wines, with OAV > 1, contributing substantial fruity aromas. The use of clarified juice in the white wine fermenter reduces esterase activity, while the lower fermentation temperature significantly increased the synthesis of ester compounds, particularly ethyl esters. In this study, DTSF wines exhibited 1.67-fold higher ethyl ester content and 1.19 times higher acetate ester content compared to the control.

We also compared the impact of modern winemaking techniques—carbonic maceration, flash détente, and saignée—on ester promotion (Table S6). The results indicate that the DTSF process enhances the synthesis of ethyl esters and total esters similarly to carbonic

Table 2

CIElab chromatic parameters of Marselan wines produced by Dual-Temperature Dual-State Fermentation (DTSF) and traditional fermentation (CK) after malolactic fermentation.

Color parameters	CK	DTSF	Significance[a]
L^*	52.98 ± 0.10	38.09 ± 0.10	***
a^*	43.94 ± 0.05	57.9 ± 0.04	***
b^*	4.77 ± 0.01	6.94 ± 0.01	***
C^*ab	44.2 ± 0.05	58.31 ± 0.04	***
hue	1.46 ± 0	1.45 ± 0	NS

[a] ***, NS: Significant at $p \leq 0.001$, or not significant, respectively, Student's t-test.

maceration (González-Arenzana et al., 2020; Tong et al., 2023) and outperforms flash détente and saignée. While flash détente strongly promotes acetate esters (Ntuli et al., 2022; Tong et al., 2024), saignée has a weaker effect on ester enhancement (Shi et al., 2023; Teng et al., 2020). Notably, DTSF requires only an additional white wine fermenter, making it more cost-effective and easier to implement compared to carbonic maceration (requiring CO_2 injection) and flash détente (requiring heating and vacuum systems).

3.4. Color parameters and sensory properties

The color parameters of the wines are presented in Table 2 and Fig. 4A. The DTSF wines exhibited lower $L*$ values indicating higher color intensity. The values of $a*$, $b*$, and $C*$ were higher in DTSF wines, which showed a stronger red-yellow hue and chromaticity compared to control wines. The superior color performance of DTSF wines can be attributed to three key factors. First, DTSF wines contain higher levels of derived anthocyanins, resulting in deeper colouration and greater color stability. The higher concentrations of vitisin A and vitisin B in DTSF wines contribute to an orange-red hue, while higher levels of flavanyl-pyranoanthocyanins and acetaldehyde-mediated polymeric pigments produce a red-purple color (Zhang, Jeffery, et al., 2022). This observation aligns with the noted increase in the red and yellow hues ($a*$, $b*$) in DTSF wines. Second, DTSF wines have higher levels of copigments (CPs), primarily flavanols and phenolic acids, which enhance copigmentation (Wu et al., 2024). This phenomenon results in a hyperchromic effect and bathochromic shift, contributing 30–50 % of the color intensity in young wines (Heras-Roger et al., 2016; Trouillas et al., 2016). Finally, the lower pH of the DTSF wines promotes the conversion of the colorless hemiketal (carbinol pseudobase) form to the red flavylium cation (He et al., 2012; X.-K. Zhang et al., 2022). pH-dependent equilibrium calculations revealed a higher percentage of red flavylium cations and a lower percentage of colorless hemiketal in DTSF wines, indicating a more pronounced red hue (Table S7).

Sensory evaluation by an experienced panel confirmed that DTSF wines exhibited a more intense fresh fruit aroma, consistent with their higher ester levels (section 3.3). Specifically, ethyl acetate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, and isoamyl acetate contributed apple, banana, and pineapple-like aromas, all exceeding their perception thresholds (OAV > 1), which showed 1.2–1.5-fold higher concentrations in DTSF wines compared to controls (Table S4). While β-damascenone may also contribute to fruit aroma, its effect is likely to be secondary to the dominant role of esters, as evidenced by no significant floral aroma differences. Notably, although significant differences were observed in terpenoids, norisoprenoids, and C6/C9 compounds between the two groups, these compounds failed to translate into green and floral aromas sensory differences. This suggests that the DTSF process primarily enhances fermentation-derived aromas while minimally affecting grape berry-derived aromas. Additionally, DTSF wines demonstrated a stronger body and reduced bitterness, although these differences were not statistically significant. This aligns with the absence of significant differences in total condensed tannin content between DTSF and control wines (Table S5), indicating that the DTSF process effectively extracts condensed tannins without compromising mouthfeel.

4. Conclusions

This study investigated the chemical and sensory characteristics of industrially produced 'Marselan' wine using dual-temperature dual-state fermentation (DTSF) technique. Compared to traditional fermentation, DTSF significantly enhanced fresh fruit aromas, attributed to low-temperature clarified juice fermentation, which promoted ester production and reduced the evaporation loss of volatile compounds. The DTSF wine also exhibited a more intense color due to higher levels of derived anthocyanins and copigments, as well as a lower pH. However, the process had minimal impact on tannin extraction, resulting in no significant difference in mouthfeel compared to the control wine.

The DTSF technique offers a versatile solution to the traditional trade-off between high-temperature fermentation (favoring phenolic extraction) and low-temperature fermentation (promoting ester formation). By adjusting parameters such as the number of cycles, separation time, and juice volume, winemakers can tailor the sensory profile of red wines to meet consumer preferences for aromatic and visually appealing products. DTSF also has limitations, including potential challenges in scalability across different grape varieties. Future research would investigate the application of DTSF to other varieties, assess its economic feasibility, and explore its long-term effects on wine stability and sensory evolution.

CRediT authorship contribution statement

Xuechen Yao: Writing – original draft, Visualization, Methodology,

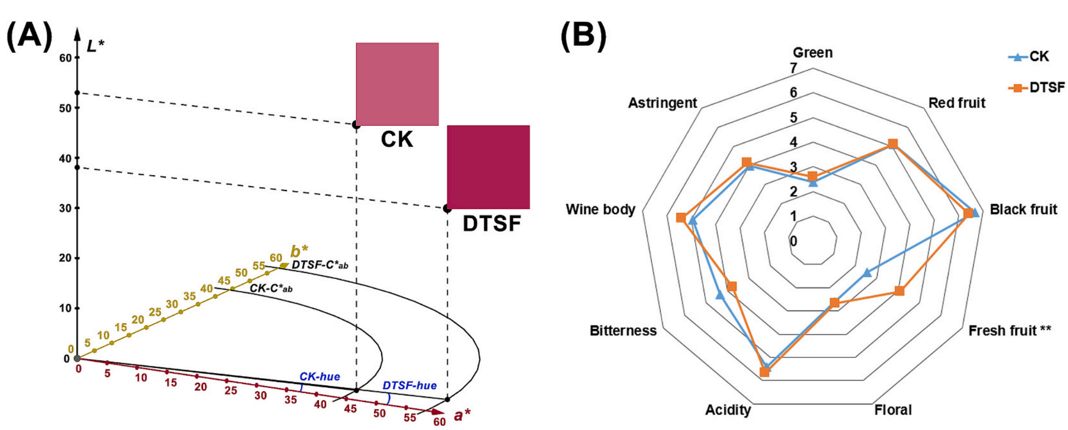

Fig. 4. Color (A) and sensory attributes (B) of CK and DTSF wines at the end of malolactic fermentation. Note: ** indicate significance at $p \leq 0.01$.

Investigation, Formal analysis. **Haoen Cai:** Visualization, Methodology, Investigation. **Jiayi Kou:** Visualization, Investigation. **Yunxue Xie:** Visualization, Investigation. **Jin Li:** Resources. **Penghui Zhou:** Resources. **Fei He:** Supervision, Conceptualization. **Changqing Duan:** Writing – review & editing, Supervision, Conceptualization. **Qiuhong Pan:** Writing – review & editing, Supervision, Conceptualization. **Mengyao Qi:** Writing – review & editing, Supervision, Conceptualization. **Yibin Lan:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102447.

Data availability

Data will be made available on request.

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